(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 March 2005 (03.03.2005)

PCT

(10) International Publication Number
WO 2005/019252 A2

(51) International Patent Classification[7]: C07K 14/37, C12N 15/62, A61K 49/00, C12N 15/12

(21) International Application Number: PCT/US2004/016252

(22) International Filing Date: 20 May 2004 (20.05.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/472,196 22 May 2003 (22.05.2003) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,

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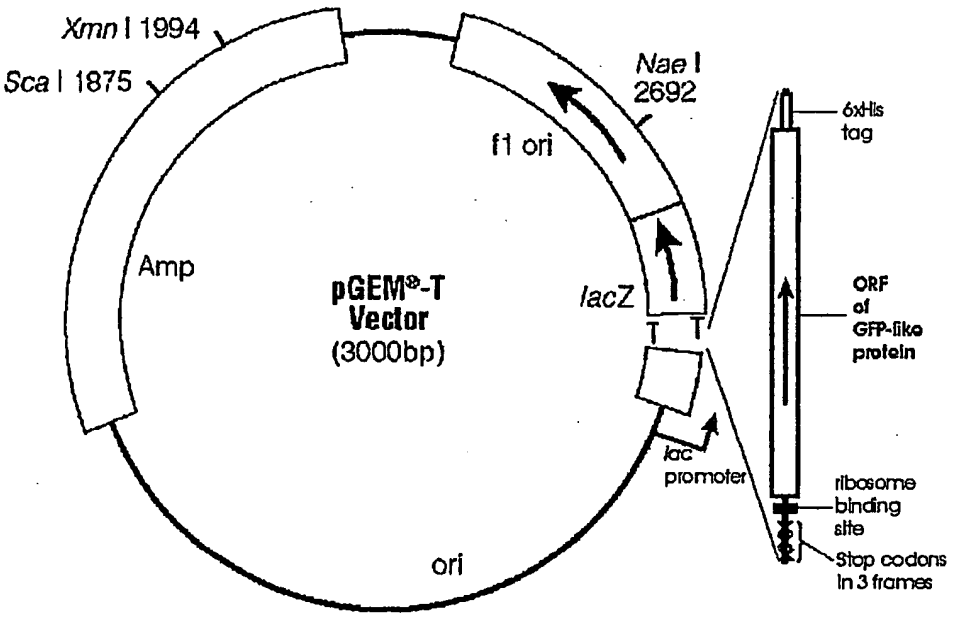
(54) Title: NOVEL FLUORESCENT AND COLORED PROTEINS, AND POLYNUCLEOTIDES THAT ENCODE THESE PROTEINS



(57) Abstract: The subject invention provides new fluorescent and/or colored proteins, and polynucleotide sequences that encode these proteins. The subject invention further provides materials and methods useful for expressing these detectable proteins in biological systems.

WO 2005/019252 A2

FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DESCRIPTION

NOVEL FLUORESCENT AND COLORED PROTEINS, AND POLYNUCLEOTIDES THAT ENCODE THESE PROTEINS

Government Support

The subject matter of this application has been supported in part by U.S. Government Support under NIH RO1 GM066243-01. Accordingly, the U.S. Government has certain rights in this invention.

Cross-Reference to a Related Application

This application claims the benefit of U.S. provisional patent application Serial No. 60/472,196, filed May 20, 2003.

Field of the Invention

The present invention relates to novel fluorescent and colored proteins, and their use. These materials and methods are particularly advantageous for labeling and detection technology. Specifically, exemplified are novel colored and/or fluorescent proteins, and mutants thereof, isolated from marine organisms. These new proteins offer a wider array of colors and biochemical features compared to existing wild-type green fluorescent protein (GFP) or its modified variants utilized in current labeling and detection technology.

Background of the Invention

Genetic markers are important for monitoring gene expression and tracking movement of proteins in cells. Markers have been extensively used for monitoring biological activity of genetic elements such as promoters, enhancers and terminators, and other aspects of gene regulation in numerous biological systems. Over the years numerous marker genes have been developed and utilized widely in molecular and genetic studies aimed at the identification, isolation and characterization of genetic regulatory elements and genes, and the development of gene transfer techniques.

In general, markers can be grouped into selectable markers and reporter markers. Selectable markers are typically enzymes with catalytic capability to

convert chemical substrates usually harmful to host cells into non-toxic products, thus providing transformed host cells a conditionally selectable growth advantage under selective environment and allowing the recovery of stable transformants after transformation. A number of commonly used selectable markers include those that confer resistance characteristics to antibiotics (Gritz and Davies 1983; Bevan *et al.*, 1983) and herbicides (De Block *et al.*, 1987), and those with enzymatic activity to detoxify metabolic compounds that can adversely affect cell growth (Joersbo and Okkels 1996).

Reporter markers are compounds that provide biochemically assayable or identifiable activities. Reporter markers have been widely used in studies to reveal biological functions and modes of action of genetic elements such as promoters, enhancers, terminators, and regulatory proteins including signal peptides, transcription factors and related gene products. Over the years, several reporter markers have been developed for use in both prokaryotic and eukaryotic systems, including β-galactosidase (LacZ) (Stanley and Luzio 1984), β-glucuronidase (GUS) (Jefferson *et al.*, 1987; U.S. Patent No. 5,268,463), chloramphenicol acetyltransferase (CAT) (Gorman *et al.*, 1982), green fluorescent protein (GFP) (Prasher *et al.*, 1992; U.S. Patent No. 5,491,084) and luciferase (Luc) (Ow *et al.*, 1986).

Among reporter markers, GUS offers a sensitive and versatile reporting capability for gene expression in plants. β-glucuronidase or GUS, encoded by the *uid*Å gene from *Escherichia coli*, catalyzes the conversion of several colorigenic and fluorogenic glucorogenic substrates such as p-nitrophenyl β-D-glucuronide and 4-methylumbelliferyl β-D-glucuronide into easily detectable products. GUS activity can be measured by highly sensitive colorimetric and fluorimetric methods (Jefferson *et al.*, 1987). However, the GUS assay often requires total destruction of the sample tissues or exposure of sample tissues to phytotoxic chemical substrates. This prevents repeated use of the same sample tissue for continuous expression analysis and precludes the recovery of transformants from analyzed materials.

Recently, GFP isolated from the Pacific Northwest jellyfish (*Aequorea victoria*) has become an important reporter marker for non-destructive analysis of gene expression. GFP fluoresces *in vivo* by receiving light energy without the involvement of any chemical substrates. Thus, GFP is especially suitable for real

time and continuous monitoring of temporal and spatial control of gene expression and protein activities without any physical damage to assay samples.

The gene for GFP has been cloned and used as a reporter gene, which can be expressed as a functional transgene in living organisms, marking the organisms with fluorescent color and thus allowing detection of those organisms. Accordingly, GFP has become a versatile fluorescent marker for monitoring a variety of physiological processes, visualizing protein localization and detecting the expression of transferred genes in various living systems, including bacteria, fungi, and mammalian tissues.

This *in vivo* labeling and detection technology was originally based on a single fluorescent protein: the green fluorescent protein from *Aequorea victoria*. Numerous modifications have been made to alter the spectral properties of GFP to provide for significant enhancement in fluorescence intensity (Prasher *et al.*, 1992; Cubitt *et al.*, 1995, Heim *et al.*, 1994, 1995; Cormack *et al.*, 1996; U.S. Patent No. 5,804,387). In addition, GFP genes have been modified to contain more silent base mutations that correspond to codon-usage preferences in order to improve its expression efficacy, making it a reporter gene in both animal and plant systems (U.S. Patent Nos. 5,874,304; 5,968,750; and 6,020,192).

In addition to GFP, there are now a number of other fluorescent proteins, substantially different from GFP, which are being developed into biotechnology tools. Most prominent of these proteins is the red fluorescent protein DsRed. See, for example, Labas, Y. A., N. G. Gurskaya, Y. G. Yanushevich, A. F. Fradkov, K. A. Lukyanov, S. A. Lukyanov and M. V. Matz. (2002) "Diversity and evolution of the green fluorescent protein family" *Proc Natl Acad Sci USA* 99:4256-4261 and Matz, M. V., K. A. Lukyanov and S. A. Lukyanov (2002) "Family of the green fluorescent protein: journey to the end of the rainbow" *Bioessays* 24: 953-959.

Labeling technologies based on GFP and related proteins have become indispensable in such areas as basic biomedical research, cell and molecular biology, transgenic research and drug discovery. The number of PubMed records containing the phrase "green fluorescent protein" exceeds 5500 only within the last three years. Demand for labeling and detection based on the fluorescent protein technology is large and steady.

Currently, there are very few known natural pigments essentially encoded by a single gene, wherein both the substrate for pigment biosynthesis and the necessary

catalytic moieties are provided within a single polypeptide chain. The limited availability of fluorescent marker proteins makes the current technology based on fluorescent proteins very expensive, rendering it unaffordable and inaccessible to many mid-size (or smaller) companies that are interested in using the technology. Therefore, there is a need for less expensive, readily available fluorescent and/or colored materials.

Brief Summary of the Invention

The subject invention provides new fluorescent and/or colored proteins, and polynucleotide sequences that encode these proteins. The subject invention further provides materials and methods useful for expressing these detectable proteins in biological systems.

In specific embodiments, the subject invention provides a red fluorescent protein from *Scolymia cubensis* scubRFP, featuring rapid conversion from immature green to mature red form under UV-A light; and three fluorescent proteins from *Montastraea cavernosa*, namely g5.2 (cyan), mc6 (green) and R7 (green) proteins. The invention also includes proteins substantially similar to, or mutants or variants of, the exemplified proteins.

Another aspect of the subject invention pertains to polynucleotide sequences that encode the detectable proteins of the present invention. In one embodiment, the present invention provides polynucleotide constructs comprising cDNA encoding novel colored and/or fluorescent proteins and mutants thereof.

The subject invention also provides proteins from *Acropora* ("staghorn corals") and *Agarica fragilis* ("fragile saucer coral"), as well as polynucleotides encoding these proteins.

In one embodiment, the invention provides nucleotide sequences of the inserts in pGEM-T vector (Promega), the conceptual translations of these inserts, and special properties of purified protein products.

The proteins and polynucleotides of the present invention can be used as described herein as colored and/or fluorescent (detectable) labels in a variety of ways, including but not limited to, as reporter genes for monitoring gene expression in living organisms, as protein tags for tracing the location of proteins within living cells and organisms, as reporter molecules for engineering various protein-based biosensors,

and as genetically encoded pigments for modifying color and/or fluorescence of living organisms or their parts.

In a specific embodiment, the proteins of the subject invention can be used in molecular fluorescent tagging whereby the coding region of a protein of interest is fused with the coding region for a fluorescent protein of the subject invention. The product of such a gene shows the functional characteristics of the protein of interest, but bears the fluorescent label allowing tracing its movements.

Advantageously, the present invention provides proteins and polynucleotides to improve on the current technology of labeling and detection by offering a wider choice of colors and biochemical features never before provided by GFP and its modified variants.

Brief Description of the Figures

Figure 1 shows design of bacterial expression constructs for the proteins of interests of the present invention.

Figure 2 shows the bacterial colonies expressing genes described in the present invention (cyan, green and red) under UV-A light. The bacterial colonies affected by the expression show red and greenish color and fluorescent appearance. These bacterial colonies are normally non-fluorescent under UV-A light.

Figure 3A-3B shows maturation of scubRFP under low-intensity UV-A light, resulting in conversion from a green-emitting form (emission maximum 520 nm) into red-emitting form (emission maximum 575 nm). Figure 3A is a graph showing the change in ratio or emission amplitudes of 520 and 575 nm. Figure 3B graph shows changes in the emission spectra.

Figure 4 shows the excitation and emission spectra of A. aculeus 1-1 (green).

Figure 5 shows the excitation and emission spectra of A. aculeus 1-2 (green).

Figure 6 shows the excitation and emission spectra of A. aculeus 2-1 (green).

Figure 7 shows the excitation and emission spectra of A. aculeus 2-2 (green).

Figure 8 shows the excitation and emission spectra of A. aculeus 3-1 (green).

Figure 9 shows the excitation and emission spectra of A. millepora 8-2 (cyan).

Figure 10 shows the excitation and emission spectra of A. millepora 9-1 (green).

Figure 11 shows the excitation and emission spectra of A. millepora 9-2 (green).

Figure 12 shows the excitation and emission spectra of A. millepora 10-1 (green).

Figure 13 shows the excitation and emission spectra of A. millepora 10-2 (cyan).

Figure 14 shows the excitation and emission spectra of A. millepora 11-1 (green).

Figure 15 shows the excitation and emission spectra of A. millepora 12-1 (red).

Figure 16 shows the excitation and emission spectra of A. nobilis 15-1 (cyan).

Figure 17 shows the excitation and emission spectra of A. nobilis 16-1 (cyan).

Figure 18 shows the excitation and emission spectra of A. nobilis 17-1 (green).

Figure 19 shows the excitation and emission spectra of Agaricia fragilis 1 (green).

Figure 20 shows the excitation and emission spectra of Agaricia fragilis 2 (green).

Figure 21 shows the excitation and emission spectra of Agaricia fragilis 3 (green).

Figure 22 shows the excitation and emission spectra of Agaricia fragilis 4 (cyan).

Figure 23 shows the excitation and emission spectra of Agaricia fragilis 5 (green).

Figure 24 shows the excitation and emission spectra of Agaricia fragilis 6 (green).

Figure 25 shows the excitation and emission spectra of Agaricia fragilis 8 (cyan).

Figure 26 shows the excitation and emission spectra of A. aculeus 5-2 (chromoprotein).

Figure 27 shows the excitation and emission spectra of A. aculeus 6-1 (chromoprotein).

Figure 28 shows the excitation and emission spectra of A. hyacinthus 7-1 (chromoprotein).

Figure 29 shows the excitation and emission spectra of A. millepora 14-1 (chromoprotein).

Brief Description of the Sequences

SEQ ID NO:1 is the 5' heel of an upstream primer used according to the subject invention.

SEQ ID NO:2 is the 5' heel of a downstream primer used according to the subject invention.

SEQ ID NO:3 is the open reading frame of the cDNA encoding the g5.2 (cyan) protein of interest from *Montastraea cavernosa.*

SEQ ID NO:4 is the open reading frame of the cDNA encoding the mc6 (green) protein of interest from *Montastraea cavernosa.*

SEQ ID NO:5 is the open reading frame of the cDNA encoding the R7 (green) protein of interest from *Montastraea cavernosa.*

SEQ ID NO:6 is the open reading frame of the cDNA encoding the scubRFP protein of interest from *Scolymia cubensis.*

SEQ ID NO:7 is the amino acid sequence encoded by SEQ ID NO:3.

SEQ ID NO:8 is the amino acid sequence encoded by SEQ ID NO:4.

SEQ ID NO:9 is the amino acid sequence encoded by SEQ ID NO:5.

SEQ ID NO:10 is the amino acid sequence encoded by SEQ ID NO:6.

SEQ ID NO:11 is the bacterial expression construct for the g5.2 (cyan) protein of interest from *Montastraea cavernosa.*

SEQ ID NO:12 is the bacterial expression construct for the mc6 (green) protein of interest from *Montastraea cavernosa.*

SEQ ID NO:13 is the bacterial expression construct for the R7 (green) protein of interest from *Montastraea cavernosa.*

SEQ ID NO:14 is the bacterial expression construct for the scubRFP protein of interest from *Scolymia cubensis.*

SEQ ID NO:15 is the amino acid sequence encoded by SEQ ID NO:11.

SEQ ID NO:16 is the amino acid sequence encoded by SEQ ID NO:12.

SEQ ID NO:17 is the amino acid sequence encoded by SEQ ID NO:13.

SEQ ID NO:18 is the amino acid sequence encoded by SEQ ID NO:14.

SEQ ID NO:19 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 1-1 in pGEM-T).

SEQ ID NO:20 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 1-2 in pGEM-T).

SEQ ID NO:21 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 2-1 in gGEM-T).

SEQ ID NO:22 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 2-2 in pGEM-T).

SEQ ID NO:23 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 3-1 in pGEM-T).

SEQ ID NO:24 is the nucleotide sequence insert of the subject invention (Acropora aculeus 5-2 in pGEM-T).

SEQ ID NO:25 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora aculeus 6-1 in pGEM-T).

SEQ ID NO:26 is the nucleotide sequence insert of the subject invention (Acropora hyacinthus 7-1 in pGEM-T).

SEQ ID NO:27 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention Acropora millepora 8-2 in pGEM-T).

SEQ ID NO:28 i is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 9-1 in pGEM-T).

SEQ ID NO:29 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 9-2 in pGEM-T).

SEQ ID NO:30 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 10-1 in pGEM-T).

SEQ ID NO:31 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 10-2 in pGEM-T).

SEQ ID NO:32 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 11-1 in pGEM-T).

SEQ ID NO:33 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 12-1 in pGEM-T).

SEQ ID NO:34 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora millepora 14-1 in pGEM-T).

SEQ ID NO:35 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora nobilis 15-1 in pGEM-T).

SEQ ID NO:36 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora nobilis 16-1 in pGEM-T).

SEQ ID NO:37 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Acropora nobilis 17-1 in pGEM-T).

SEQ ID NO:38 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 1 in pGEM-T).

SEQ ID NO:39 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 2 in pGEM-T).

SEQ ID NO:40 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 3 in pGEM-T).

SEQ ID NO:41 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 4 in pGEM-T).

SEQ ID NO:42 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 5 in pGEM-T).

SEQ ID NO:43 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 6 in pGEM-T).

SEQ ID NO:44 is the nucleotide sequence of an insert in the pGEM-T vector, according to subject invention (Agaricia fragilis 8 in pGEM-T).

SEQ ID NO:45 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 1-1 in pGEM-T.

SEQ ID NO:46 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 1-2 in pGEM-T.

SEQ ID NO:47 is the amino aid sequence of a protein of the subject invention as expessed by the following construct: Acropora aculeus 2-1 in pGEM-T.

SEQ ID NO:48 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 2-2 in pGEM-T.

SEQ ID NO:49 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 3-1 in pGEM-T.

SEQ ID NO:50 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 5-2 in pGEM-T.

SEQ ID NO:51 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora aculeus 6-1 in pGEM-T.

SEQ ID NO:52 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora hyacinthus 7-1 in pGEM-T.

SEQ ID NO:53 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora millepora 8-2 in pGEM-T.

SEQ ID NO:54 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora millepora 9-1 in pGEM-T.

SEQ ID NO:55 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora millepora 9-2 in pGEM-T.

SEQ ID NO:56 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora millepora 10-1 in pGEM-T.

SEQ ID NO:57 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora millepora 10-2 in pGEM-T.

SEQ ID NO:58 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora millepora 11-1 in pGEM-T.

SEQ ID NO:59 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora millepora 12-1 in pGEM-T.

SEQ ID NO:60 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora millepora 14-1 in pGEM-T.

SEQ ID NO:61 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora nobilis 15-1 in pGEM-T.

SEQ ID NO:62 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora nobilis 16-1 in pGEM-T.

SEQ ID NO:63 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Acropora nobilis 17-1 in pGEM-T.

SEQ ID NO:64 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Agaricia fragilis 1 in pGEM-T.

SEQ ID NO:65 is the amino aid sequence of a protein of the subject invention as expressed by the following construct: Agaricia fragilis 2 in pGEM-T.

SEQ ID NO:66 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Agaricia fragilis 3 in pGEM-T.

SEQ ID NO:67 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Agaricia fragilis 4 in pGEM-T.

SEQ ID NO:68 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Agaricia fragilis 5 in pGEM-T.

SEQ ID NO:69 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Agaricia fragilis 6 in pGEM-T.

SEQ ID NO:70 is the amino aid sequence of a protein of the subject invention as encoded by the following construct: Agaricia fragilis 8 in pGEM-T.

SEQ ID NO:71 is the coding region of the construct of SEQ ID NO:45.

SEQ ID NO:72 is the coding region of the construct of SEQ ID NO:46.

SEQ ID NO:73 is the coding region of the construct of SEQ ID NO:47.

SEQ ID NO:74 is the coding region of the construct of SEQ ID NO:48.

SEQ ID NO:75 is the coding region of the construct of SEQ ID NO:49.

SEQ ID NO:76 is the coding region of the construct of SEQ ID NO:50.

SEQ ID NO:77 is the coding region of the construct of SEQ ID NO:51.

SEQ ID NO:78 is the coding region of the construct of SEQ ID NO:52.

SEQ ID NO:79 is the coding region of the construct of SEQ ID NO:53.

SEQ ID NO:80 is the coding region of the construct of SEQ ID NO:54.

SEQ ID NO:81 is the coding region of the construct of SEQ ID NO:55.

SEQ ID NO:82 is the coding region of the construct of SEQ ID NO:56.

SEQ ID NO:83 is the coding region of the construct of SEQ ID NO:57.

SEQ ID NO:84 is the coding region of the construct of SEQ ID NO:58.

SEQ ID NO:85 is the coding region of the construct of SEQ ID NO:59.

SEQ ID NO:86 is the coding region of the construct of SEQ ID NO:60.

SEQ ID NO:87 is the coding region of the construct of SEQ ID NO:61.

SEQ ID NO:88 is the coding region of the construct of SEQ ID NO:62.

SEQ ID NO:89 is the coding region of the construct of SEQ ID NO:63.

SEQ ID NO:90 is the coding region of the construct of SEQ ID NO:64.

SEQ ID NO:91 is the coding region of the construct of SEQ ID NO:65.

SEQ ID NO:92 is the coding region of the construct of SEQ ID NO:66.

SEQ ID NO:93 is the coding region of the construct of SEQ ID NO:67.

SEQ ID NO:94 is the coding region of the construct of SEQ ID NO:68.

SEQ ID NO:95 is the coding region of the construct of SEQ ID NO:69.

SEQ ID NO:96 is the coding region of the construct of SEQ ID NO:70.

Detailed Description of the Invention

The present invention provides novel fluorescent and colored proteins isolated from marine organisms other than *Aequorea Victoria*. In a particularly preferred embodiment, these proteins are red fluorescent proteins featuring rapid conversion from immature green to mature red under UV-A light. Specifically exemplified herein are scubRFP from *Scolymia cubensis*; and g5.2 (cyan), mc6 (green) and R7 (green) proteins, from *Montastraea cavernosa*.

The subject invention further provides polynucleotide sequences encoding these proteins. These polynucleotide sequences include open reading frames encoding the specific exemplified detectable proteins, as well as expression constructs for expressing these proteins, for example, in bacterial hosts.

The proteins of the present invention can be readily, expressed by any one of the recombinant technology methods known to those skilled in the art having the benefit of the instant disclosure. The preferred method will vary depending upon many factors and considerations, including the host, and the cost and availability of materials and other economic considerations. The optimum production procedure for a given situation will be apparent to those skilled in the art having the benefit of the current disclosure.

The subject invention also concerns cells transformed with a polynucleotide of the present invention comprising a nucleotide sequences encoding a novel detectable protein. These cells may be prokaryotic or eukaryotic, plant or animal. In one embodiment, animals, such as fish, are transformed to provide them with a unique color or ability to fluoresce. Polynucleotides providing the markers of the present invention are stable in a diverse range of hosts, including prokaryotic and eukaryotic organisms, and the translation products are fully functional and capable of providing assayable characteristics.

In another embodiment, the present invention provides methods to synthesize colored and fluorescent proteins in a recombinant cell.

In a specific embodiment, the proteins of the subject invention can be used in molecular fluorescent tagging whereby the coding region of a protein of interest is fused with the coding region for a fluorescent protein of the subject invention. The

product of such a gene shows the functional characteristics of the protein of interest, but bears the fluorescent label allowing tracing its movements. See, for example, Eichinger, L., S. S. Lee and M. Schleicher (1999) "Dictyostelium as model system for studies of the actin cytoskeleton by molecular genetics" *Microsc Res Tech* 47:124-134; Falk, M. M. and U. Lauf (2001) "High resolution, fluorescence deconvolution microscopy and tagging with the autofluorescent tracers CFP, GFP, and YFP to study the structural composition of gap junctions in living cells" Microsc Res Tech 52:251-262; Kallal, L. and J. L. Benovic (2000) "Using green fluorescent proteins to study G-protein-coupled receptor localization and trafficking" Trends Pharmacol Sci 21:175-180; and Laird, D. W., K. Jordan, T. Thomas, H. Qin, P. Fistouris and Q. Shao (2001) "Comparative analysis and application of fluorescent protein-tagged connexins" Microsc Res Tech 52:263-272.

In a further embodiment, the subject invention concerns polynucleotides comprising an in-frame fusion of nucleotide sequences encoding multiple genetic markers. In one embodiment, the polynucleotides encode the genetic markers GUS, and a detectable protein of the subject invention.

The subject invention helps to provide a more abundant and diverse collection of proteins, which can be used in place of a GFP protein, such that new proteins are readily available for commercial exploitation by small companies that cannot take advantage of the current technology for financial reasons.

Definitions

As used herein, the terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide, ribonucleotide, or a mixed deoxyribonucleotide and ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally-occurring nucleotides.

As used herein, "a vector" is a DNA sequence having the elements necessary for the transcription/translation of a gene. Such elements would include, for example, promoters. Various classes of promoters are well known in the art and can be obtained commercially or assembled from the sequences and methods, which are also well known in the art. A number of vectors are available for expression and/or

cloning, and include, but are not limited to, pBR322, pUC series, M13 series, and pBLUESCRIPT vectors (Stratagene, La Jolla, CA).

As used herein, the term "expression construct" refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. As used herein, the term "operably linked" refers to a juxtaposition of the components described wherein the components are in a relationship that permits them to function in their intended manner. In general, operably linked components are in contiguous relation.

Detectable Proteins

The subject invention provides novel fluorescent and/or colored proteins. These proteins are exemplified by scubRFP from *Scolymia cubensis* (SEQ ID NO. 7); and g5.2 (cyan) (SEQ ID NO. 8), mc6 (green) (SEQ ID NO. 9) and R7 (green) (SEQ ID NO. 10) proteins, from *Montastraea cavernosa*.

The novel colored and fluorescent proteins of the present invention can be detected using standard long-wave UV light sources or, preferably, optical designs appropriate for detecting agents with the excitation/emission characteristics of the proteins exemplified herein (see, for example, Figures 2-29). These proteins are referred to herein as "detectable proteins" or "marker proteins." The interaction of two or more residues of the protein and external agents such as molecular oxygen give rise to the colored and/or fluorescent feature of the proteins.

Advantageously, the use of these proteins facilitate real-time detection *in vivo*, a substrate is not required, and the relatively small size make the proteins very advantageous.

Substitution of amino acids other than those specifically exemplified or naturally present in the genetic marker proteins of the invention are also contemplated within the scope of the present invention. Such substitutions will create "variant proteins" within the scope of the subject invention. Variants and fragments preferably have emission and excitation maxima within 10 nm of the values shown in Figures 2-29. For example, non-natural amino acids can be substituted for the amino acids of the marker proteins, so long as a marker protein having the substituted amino acids retains its ability to be detected through fluorescence and/or color. Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline,

hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ-amino butyric acid, ε-amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, τ-butylglycine, τ-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form. Allelic variants of a protein sequence of a detectable protein used in the present invention are also encompassed within the scope of the invention.

Amino acids can be generally categorized in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby a marker protein having an amino acid of one class is replaced with another amino acid of the same class fall within the scope of the subject invention so long as a marker protein having the substitution still is detectable Table 1 below provides a listing of examples of amino acids belonging to each class.

Table 1.

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

Polynucleotides

cDNA sequences encoding the proteins of the present invention are provided. Polynucleotides of the present invention can be composed of either RNA or DNA. Preferably, the polynucleotides are composed of DNA. The subject invention also

encompasses those polynucleotides that are complementary in sequence to the polynucleotides disclosed herein.

Specifically exemplified are DNA sequences that encode for scubRFP from *Scolymia cubensis*; and g5.2 (cyan), mc6 (green) and R7 (green) proteins, from *Montastraea cavernosa*. These DNA sequences are set forth in SEQ. ID NOS. 3-6.

Sequences of the subject invention may utilize codons preferred for expression by the selected host strains. These sequences may also have sites for cleavage by restriction enzymes, and/or initial, terminal, or intermediate DNA sequences which facilitate construction of readily expressed vectors.

Because of the degeneracy of the genetic code, a variety of different polynucleotide sequences can encode the detectable proteins of the present invention. In addition, it is well within the skill of a person trained in the art to create alternative polynucleotide sequences encoding the same, or essentially the same, detectable proteins of the subject invention. These variant or alternative polynucleotide sequences are within the scope of the subject invention. As used herein, references to "essentially the same" sequence refers to sequences which encode amino acid substitutions, deletions, additions, or insertions which do not eliminate the detectability of the polypeptide encoded by the polynucleotides of the present invention. Allelic variants of the nucleotide sequences encoding a genetic marker protein of the invention are also encompassed within the scope of the invention.

The subject invention also concerns variants of the polynucleotides of the present invention that encode detectable proteins. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted. The nucleotides that can be substituted for natural nucleotides of DNA have a base moiety that can include, but is not limited to, inosine, 5-fluorouracil, 5-bromouracil, hypoxanthine, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified and includes, but is not limited to, arabinose, xylulose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

Polynucleotides and polypeptides of the subject invention can also be defined in terms of more particular identity and/or similarity ranges with those exemplified herein. The sequence identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used.

The subject invention also contemplates those polynucleotide molecules having sequences that are sufficiently homologous with the polynucleotide sequences exemplified herein so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis *et al.* 1982). As used herein, "stringent" conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25 C below the melting temperature (Tm) of the DNA hybrid in 6x SSPE, 5x Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature, Tm, is described by the following formula (Beltz *et al.*, 1983):

Tm=81.5 C+16.6 Log[Na+]+0.41(%G+C)-0.61(% formamide)-600/length of duplex in base pairs.

Washes are typically carried out as follows:

(1) Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).

(2) Once at Tm-20 C for 15 minutes in 0.2x SSPE, 0.1% SDS (moderate stringency wash).

The polynucleotide sequences include the DNA strand sequence that is transcribed into RNA and the strand sequence that is complementary to the DNA strand that is transcribed. The polynucleotide sequences also include both full-length sequences as well as shorter sequences derived from the full-length sequences. The polynucleotide sequence includes both the sense and antisense strands either as individual strands or in the duplex.

Recombinant Hosts

Polynucleotide molecules containing DNA sequences encoding the colored and/or fluorescent proteins of the present invention can be introduced into a variety of host cells including bacterial cells, yeast cells, fungal cells, plant cells and animal cells. Methods by which the exogenous genetic material can be introduced into such host cells are well known in the art.

In one embodiment, the invention provides a bacteria cell capable of expressing the novel colored and fluorescent proteins.

Plants, plant tissues, and plant cells bred to contain, or transformed with, a polynucleotide of the invention are also contemplated by the present invention. In one embodiment, the polynucleotide encodes a detectable polypeptide shown in SEQ ID NOS. 7-10, or a functional fragment or variant thereof. Plants within the scope of the present invention include monocotyledonous plants, such as rice, wheat, barley, oats, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, grasses, and millet; and dicotyledonous plants, such as peas, alfalfa, tomato, melon, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple trees, grape, cotton, sunflower, and lettuce; and conifers. Techniques for transforming plant cells with a gene are known in the art and include, for example, *Agrobacterium* infection, biolistic methods, electroporation, calcium chloride treatment, *etc.* Transformed cells can be selected, redifferentiated, and grown into plants using standard methods known in the art. The progeny of any transformed plant cells or plants are also included within the scope of the present invention.

The subject invention also concerns non-human transgenic animals which have incorporated into the host cell genome a polynucleotide of the invention. Methods for producing transgenic animals, including mice, rats, pigs, sheep, cows, fish, and the like are well known in the art.

The subject invention also concerns methods for isolating transformants expressing a transgene. In one embodiment, an expression construct of the present invention comprising a transgene of interest operably linked to a nucleotide sequence encoding a detectable marker of the present invention is used to transform a cell. Methods for transforming cells are well known in the art. Transformed cells expressing the transgene are selected by identifying those cells expressing a genetic marker of the invention.

Expression Constructs

An expression construct of the invention typically comprises a structural gene sequence (encoding a protein), an antisense sequence, or other polynucleotide sequences, or a site for insertion of such sequences, operably linked to a polynucleotide of the present invention encoding a marker. The structural gene can be a gene encoding a protein from a prokaryotic or eukaryotic organism, for example, a human, mammal, insect, plant, bacteria, or virus. Proteins that can be encoded by a gene sequence include, but are not limited to, enzymes, hormones, cytokines, interleukins, receptors, growth factors, immunoglobulins, transcription factors, and *Bacillus thuringiensis* (*B.t.*) crystal toxin proteins. Sequences encoding *B.t.* proteins which have codon usage for preferential expression in plants are described in U.S. Patent Nos. 5,380,831; 5,567,862; 5,567,600; 6,013,523; and 6,015,891. An antisense sequence is a sequence wherein the RNA transcribed from the antisense sequence is at least partially complementary to RNA transcribed from a gene encoding a protein.

Expression constructs of the invention will also generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Thus, a person of ordinary skill in the art can select regulatory elements for use in, for example, bacterial host cells, yeast host cells, plant host cells, insect host cells, mammalian host cells, and human host cells. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements.

An expression construct of the invention can comprise a promoter sequence operably linked to a polynucleotide sequence encoding a marker of the invention. Promoters can be incorporated into a polynucleotide using standard techniques known in the art. Multiple copies of promoters or multiple promoters can be used in an

expression construct of the invention. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

For expression in prokaryotic systems, an expression construct of the invention can comprise promoters such as, for example, alkaline phosphatase promoter, tryptophan (trp) promoter, lambda P_L promoter, β-lactamase promoter, lactose promoter, phoA promoter, T3 promoter, T7 promoter, or tac promoter (de Boer *et al.*, 1983).

Expression constructs for use in bacteria are given in SEQ ID NOS. 11-14, and the corresponding amino acid sequences are given in SEQ ID NOS. 15-18.

If the expression construct is to be provided in a plant cell, plant viral promoters, such as, for example, the cauliflower mosaic virus (CaMV) 35S (including the enhanced CaMV 35S promoter (see, for example U.S. Patent No. 5,106,739)) or 19S promoter can be used. Plant promoters such as prolifera promoter, Ap3 promoter, heat shock promoters, T-DNA 1'- or 2'-promoter of *A. tumafaciens*, polygalacturonase promoter, chalcone synthase A (CHS-A) promoter from petunia, tobacco PR-1a promoter, ubiquitin promoter, actin promoter, alcA gene promoter, pin2 promoter (Xu *et al.*, 1993), maize WipI promoter, maize trpA gene promoter (U.S. Patent No. 5,625,136), maize CDPK gene promoter, and RUBISCO SSU promoter (U.S. Patent No. 5,034,322) can also be used. Seed-specific promoters such as the promoter from a β-phaseolin gene (of kidney bean) or a glycinin gene (of soybean), and others, can also be used. Constitutive promoters (such as the CaMV, ubiquitin, actin, or NOS promoter), tissue-specific promoters (such as the E8 promoter from tomato), developmentally-regulated promoters, and inducible promoters (such as those promoters than can be induced by heat, light, hormones, or chemicals) are contemplated for use with the polynucleotides of the invention.

For expression in animal cells, an expression construct of the invention can comprise suitable promoters that can drive transcription of the polynucleotide sequence. If the cells are mammalian cells, then promoters such as, for example, actin promoter, metallothionein promoter, NF-kappaB promoter, EGR promoter, SRE promoter, IL-2 promoter, NFAT promoter, osteocalcin promoter, SV40 early

promoter and SV40 late promoter, Lck promoter, BMP5 promoter, TRP-1 promoter, murine mammary tumor virus long terminal repeat promoter, STAT promoter, or an immunoglobulin promoter can be used in the expression construct. The baculovirus polyhedrin promoter can be used with an expression construct of the invention for expression in insect cells. Promoters suitable for use with an expression construct of the invention in yeast cells include, but are not limited to, 3-phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase promoter, metallothionein promoter, alcohol dehydrogenase-2 promoter, and hexokinase promoter.

Expression constructs of the invention may optionally contain a transcription termination sequence, a translation termination sequence, signal peptide sequence, and/or enhancer elements. Transcription termination regions can typically be obtained from the 3' untranslated region of a eukaryotic or viral gene sequence. Transcription termination sequences can be positioned downstream of a coding sequence to provide for efficient termination. Signal peptides are a group of short amino terminal sequences that encode information responsible for the relocation of an operably linked mature polypeptide to a wide range of post-translational cellular destinations, ranging from a specific organelle compartment to sites of protein action and the extracellular environment. Targeting marker gene products to an intended cellular and/or extracellular destination through the use of operably linked signal peptide sequence is contemplated for use with the polypeptides of the invention. Enhancers are cis-acting elements that increase activity of a promoter and can also be included in the expression construct. Enhancer elements are known in the art, and include, but are not limited to, the CaMV 35S enhancer element, maize shrunken-1 enhancer element, cytomegalovirus (CMV) early promoter enhancer element, and the SV40 enhancer element.

DNA sequences which direct polyadenylation of the mRNA encoded by the structural gene can also be included in the expression construct. The expression constructs of the invention can also include a polynucleotide sequence that directs transposition of other genes, *i.e.*, a transposon.

Applications

There are many ways in which the novel proteins of the subject invention can be used. In one embodiment, the proteins can be used to identify cells. In these

methods the proteins can be used to express fluorescence in a cell. One use for this method is in pre-labeling isolated cells or a population of similar cells prior to exposing the cells to an environment in which different cell types are present. Detection of fluorescence in only the original cells allows the location of such cells to be determined and compared with the total population.

A second group of methods concerns the identification of cells that have been transformed with exogenous DNA of interest. Identifying cells transformed with exogenous DNA is required in many *in vitro* procedures as well as in *in vivo* applications such as gene therapy.

In one embodiment of the subject invention, a polynucleotide sequence encoding a protein of the subject invention is fused to a DNA sequence encoding a selected protein in order to directly label the encoded protein. Expressing such a fluorescent and/or colored protein in a cell results in the production of labeled proteins that can be readily detected. This is useful in confirming that a protein is being produced by a chosen host cell. It also allows the location of the selected protein to be determined.

Cells that have been transformed with exogenous DNA can also be identified without creating a fusion protein. Here, the method relies on the identification of cells that have received a plasmid or vector that comprises at least two transcriptional or translational units. A first unit encodes and directs expression of the desired protein, while the second unit encodes and directs expression of the detectable protein. Co-expression of the detectable protein from the second transcriptional or translational unit ensures that cells containing the vector are detected and differentiated from cells that do not contain the vector.

In methods to produce fluorescent molecular weight markers, a gene sequence is generally fused to one or more DNA sequences that encode proteins having defined amino acid sequences and the fusion proteins are expressed from an expression vector. Expression results in the production of fluorescent proteins of defined molecular weight or weights that may be used as markers (following calculation of the size of the complete amino acid sequence).

Amino acid replacements that produce different color forms permit simultaneous use of multiple reporter genes. Different colored proteins can be used to identify multiple cell populations in a mixed cell culture or to track multiple cell

types, enabling differences in cell movement or migration to be visualized in real time without the need to add additional agents or fix or kill the cells.

Other options include tracking and determining the ultimate location of multiple proteins within a single cell, tissue or organism; differential promoter analysis in which gene expression from two different promoters is determined in the same cell, tissue or organism; and FACS sorting of mixed cell populations.

The techniques that can be used with spectrally separable proteins are exemplified by confocal microscopy, flow cytometry, and fluorescence activated cell sorting (FACS) using modular flow, dual excitation techniques.

In one embodiment, the subject invention concerns polynucleotides comprising an in-frame fusion of nucleotide sequences encoding multiple genetic markers. For example, a polynucleotide of the invention may comprise a first nucleotide sequence that is operably linked in-frame to a second nucleotide sequence. The polynucleotide encodes the amino acid sequences of the detectable protein and another genetic marker such that the genetic markers are in direct contact with one another, *i.e.*, where the last amino acid of the fluorescent genetic marker is immediately contiguous with the first amino acid of the other genetic marker, or they can be separated by a peptide linker sequence, for example, as described in U.S. Patent Nos. 5,891,680 and Li *et al.*, 2001, that do not substantially alter functional activity of the genetic markers.

The subject invention also concerns kits comprising in one or more containers and a polynucleotide and/or protein of the present invention.

Additional useful applications of the technology described herein include, but are not limited to, the following:

FRET – Fluorescence Resonant Energy Transfer: This technique allows observation and quantification of molecular interactions. It requires at least two fluorescent proteins of different colors. Currently the most widely used pair is CFP and YFP (mutated variants of GFP); the proteins of the subject invention may be substituted for either or both of them.

References:

1. Hanson, M. R. and R. H. Kohler. 2001. GFP imaging: methodology and application to investigate cellular compartmentation in plants. *J Exp Bot* 52: 529-539.
2. Pollok, B. A. and R. Heim. 1999. Using GFP in FRET-based applications. *Trends Cell Biol* 9: 57-60.
3. Schuttrigkeit, T. A., U. Zachariae, T. von Feilitzsch, J. Wiehler, J. von Hummel, B. Steipe and M. E. Michel-Beyerle. 2001. Picosecond time-resolved FRET in the fluorescent protein from Discosoma Red (wt-DsRed). *Chemphyschem* 2: 325-328.
4. Hillisch, A., M. Lorenz and S. Diekmann. 2001. Recent advances in FRET: distance determination in protein-DNA complexes. *Curr Opin Struct Biol* 11: 201-207.

FRAP – Fluorescence Redistribution After Photobleaching: Tthis technique quantifies the dynamics of tagged molecules or the reporter molecules themselves. It involves in photobleaching (burning out) of all the fluorescent molecules within a small area by intense excitation light and monitoring the process of fluorescence recovery within this area (due to migration of tagged molecules from adjacent areas).

References:

1. Reits, E. A. and J. J. Neefjes. 2001. From fixed to FRAP: measuring protein mobility and activity in living cells. *Nat Cell Biol* 3: E145-147.
2. Houtsmuller, A. B. and W. Vermeulen. 2001. Macromolecular dynamics in living cell nuclei revealed by fluorescence redistribution after photobleaching. *Histochem Cell Biol* 115: 13-21.

"Fluorescent timer" applications: one of the proteins exemplified herein – scubRFP – due to its natural spectroscopic properties, can be used as a reporter that changes color with time. Such reporters make it possible to estimate the time elapsed since the reporter protein was synthesized by quantifying its color. In addition, since the maturation speed (the rate of conversion from green to red) in scubRFP can be increased by UV-A light, it is possible to adjust its timing scale: experiments that need timing in shorter intervals may use appropriate background UV illumination to speed up the green-to-red conversion.

References:

1. Terskikh, A. V., A. Fradkov, A. Zaraiskiy, A. V. Kajava, M. Matz, S. Kim, I. Weissman and P. Siebert. 2000. "Fluorescent timer": Protein that changes color over time. *Molecular Biology of the Cell* 11: 648.
2. Verkhusha, V. V., H. Otsuna, T. Awasaki, H. Oda, S. Tsukita and K. Ito. 2001. An enhanced mutant of red fluorescent protein DsRed for double labeling and developmental timer of neural fiber bundle formation. *Journal of Biological Chemistry* 276: 29621-29624.

"Light-inducible fluorescence": since the red fluorescence of scubRFP can be induced by exposure to UV-A light, it is possible to use this protein as a light-inducible reporter. Such a reporter can be used for studying molecular dynamics, in a way that is analogous to FRAP (see above). A small area can be irradiated by the fluorescence-inducing light, after which the process of redistribution of active fluorescent molecules from the irradiated spot can be followed.

References:

1. Ando, R., H. Hama, M. Yamamoto-Hino, H. Mizuno and A. Miyawaki. 2002. An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein. Proceedings of the National Academy of Sciences of the United States of America 99: 12651-12656.
2. Patterson, G. H. and J. Lippincott-Schwartz. 2002. A photoactivatable GFP for selective photolabeling of proteins and cells. *Science* 297: 1873-1877.
3. Chudakov, D. M., V. V. Belousov, A. G. Zaraisky, V. V. Novoselov, D. B. Staroverov, D. B. Zorov, S. Lukyanov and K. A. Lukyanov. 2003. Kindling fluorescent proteins for precise in vivo photolabeling (vol 21, pg 191, 2003). *Nature Biotechnology* 21: 452-452.

Coloring of biological objects for decorative and other non-scientific purposes. Examples: producing decorative fish for aquariums; coloring of fur, wool and milk by means of genetic modifications of appropriate animals; and coloring of decorative plants. Such uses can be implemented by a person skilled in the art having the benefit of the teachings of the current disclosure.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 — Bacterial Expression Construct

As illustrated in Figure 1, to prepare a bacterial expression construct, the ORF of the target detectable protein was amplified by means of polymerase chain reaction (PCR), using primers corresponding to the beginning and end of the protein's ORF. The upstream primer carried a 5'-heel **ttgattgattga**aggagaaatatcATG (SEQ ID NO:1), which encoded three termination codons in three frames (bold), followed by the ribosome binding site (underlined), 6 spacer bases and initiation ATG codon.

The downstream primer encoded a 6xHis tag in place of the original termination codon (the heel sequence was 5'-tta tta gtg atg gtg atg gtg atg (SEQ ID NO:2)), to facilitate protein purification by means of metal-affinity chromatography.

The products of amplification were cloned into pGEM-T vector (Promega) using manufacturer-provided reagents and protocol. The expressing clones were identified after overnight growth of the colonies by their fluorescent appearance.

Example 2 — Additional Proteins and Polynucleotides

The subject invention also provides proteins from *Acropora* ("staghorn corals") and *Agarica fragilis* ("fragile saucer coral"), as well as polynucleotides encoding these proteins.

In one embodiment, the invention provides nucleotide sequences of the inserts in pGEM-T vector (Promega), the conceptual translations of these inserts, and special properties of purified protein products.

The vector constructs are shown in SEQ ID NOs.:19-44. The encoded proteins are shown in SEQ ID NOs.:45-70. The open reading frames encoding the

proteins of SEQ ID NOs.: 45-70 are shown in SEQ ID NOs.:71-96. The spectral characteristics of the proteins are shown in Figures 4-29.

Example 3 — Excitation and emission spectra of the detectable proteins

The excitation spectra were measured from the proteins purified after bacterial expression. The spectra are shown in Figures 2-29. Emission spectra (dotted lines) were measured using USB2000 uv-vis spectrometer (Ocean Optics), excitation spectra (solid lines) – using spectrofluorometer LS-50B (Perkin Elmer). The indicated positions of excitation and emission maxima are accurate within 5 nm.

Example 4 — Multiple Marker Constructs

There are several advantages associated with the use of fusion markers, including: 1) achievement of combined functionalities in a single transcription unit, 2) reduced usage of genetic elements, such as promoters and terminators, for expressing multiple marker genes, 3) reduced overall length of insertion sequences that may lead to increased transformation efficiency, and most importantly 4) elimination of molecular interactions between adjacent genetic elements. Such unwanted interactions are frequently encountered when multiple expression units associated with different marker genes are used simultaneously and often complicate the interpretation of expression results.

In an effort to improve marker functionality and versatility, several translational fusions between two genetic markers have been developed. Datla *et al.* (1991; U.S. Patent No. 5,639,663) created a bifunctional fusion between GUS and neomycin phosphotransferase (NPTII) to provide a biochemically assayable reporter activity and a conditionally selectable growth advantage for use in plant transformation. Another bifunctional fusion, between GUS and GFP, was also developed to provide both indicative and assayable reporter activities for monitoring transient and stable transgene expression in plant cells (Quaedvlieg *et al.*, 1998). More recently, Li *et al.* (2001) constructed a bifunctional fusion between GFP and NPTII and successfully used this marker for continuous analysis of promoter activity and transgene expression in transgenic grape plants throughout the entire process of plant development.

Small portions of a protein that provide unique functions such as protein/DNA/substrate binding activity can be inserted into another heterologous protein to create a hybrid fusion with enhanced functionality and utility. In other cases, an entire gene or protein of interest has been fused in-frame to another heterologous gene or protein to form a double fusion to provide combined functionalities. Production of multiple proteins using fusion constructs composed of two genes from transgenic plants has been demonstrated previously (U.S. Patent No. 6,455,759).

In one embodiment, the subject invention provides cells transformed with a polynucleotide of the present invention comprising an in-frame fusion of nucleotide sequences encoding multiple markers. Preferably, the polynucleotide sequence is provided in an expression construct of the invention. The transformed cell can be a prokaryotic cell, for example, a bacterial cell such as *E. coli* or *B. subtilis*, or the transformed cell can be a eukaryotic cell, for example, a plant or animal cell. Animal cells include human cells, mammalian cells, avian cells, fish cells and insect cells. Mammalian cells include, but are not limited to, COS, 3T3, and CHO cells.

Genetic markers that can be used in conjunction with the detectable proteins of the present invention are known in the art and include, for example, polynucleotides encoding proteins that confer a conditionally selective growth advantage, such as antibiotic resistance and herbicide-resistance; polynucleotides encoding proteins that confer a biochemically assayable reporter activity; and polynucleotides encoding proteins that confer an indicative reporter activity. Examples of polynucleotides encoding proteins providing antibiotic resistance include those that can provide for resistance to one or more of the following antibiotics: hygromycin, kanamycin, bleomycin, G418, streptomycin, paromomycin, and spectinomycin. Kanamycin resistance can be provided by neomycin phosphotransferase (NPTII). Examples of polynucleotides encoding proteins providing herbicide resistance include those that can provide for resistance to phosphinothricin acetyltransferase or glyphosate. Examples of genetic markers that confer assayable or indicative reporters activity that can be used in the present invention include, but are not limited to, polynucleotides encoding β-glucuronidase (GUS), β-galactosidase, chloramphenicol acetyltransferase (CAT), luciferase, nopaline synthase (NOS), and green fluorescence protein (GFP).

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Claims

We claim:

1. A protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70 or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within ± 10 nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70.

2. The protein, according to claim 1, wherein said protein has an amino acid sequence selected from the group consisting of SEQ. ID NOs: 7-10 and SEQ ID NOs: 45-70.

3. The protein, according to claim 1, wherein said protein has an amino acid sequence selected from the group consisting of SEQ. ID NOs: 7-10.

4. The protein, according to claim 1, wherein said protein has SEQ ID NO:10.

5. A polynucleotide sequence that encodes a protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70, or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within ± 10 nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70.

6. The polynucleotide sequence, according to claim 5, wherein said polynucleotide has a sequence is selected from the group consisting of SEQ. ID NOs: 3-6 and SEQ ID NOs: 71-96.

7. A polynucleotide sequence selected from the group consisting of SEQ ID NOs: 11-14 and SEQ ID NOs: 19-44.

8. A protein selected from the group consisting of the proteins expressed when a bacterium is transformed with a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 11-14 and SEQ ID NOs: 19-44.

9. Use of a protein, wherein said protein comprises a protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70, or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within ± 10 nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70, for monitoring gene expression; as a tag for tracing the location of proteins; as a reporter molecule in a protein-based biosensor; or as a pigment for modifying the color, or fluorescence, or both, of living tissue.

10. The use, according to claim 9, wherein a polynucleotide that encodes said protein is fused with a polynucleotide that encodes a protein of interest.

11. A cell transformed to express a heterologous polynucleotide wherein said heterologous polynucleotide encodes a protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70 or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within ± 10 nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70.

12. The cell, according to claim 11, wherein said cell is a plant cell.

13. The cell, according to claim 11, wherein said cell is a fish cell.

14. A polynucleotide encoding multiple markers wherein at least one of said markers is selected from the group consisting of a protein comprising an amino sequence selected from the group consisting of SEQ. ID NOs: 7-10, SEQ ID NOs: 45-70, fragments of these sequences, and variants of these sequences and fragments; wherein the amino acid sequence of a variant is at least 90% identical to at least one of said SEQ ID NOs.:7-10 and 45-70 or a fragment of one of said sequences; and wherein said fragments and said variants have emission and excitation maxima there are within ± 10 nm of the emission and excitation maxima for at least one of SEQ ID NOs.: 7-10 and SEQ ID NOs. 45-70.

15. The polynucleotide, according to claim 14, wherein one of said markers is GUS.

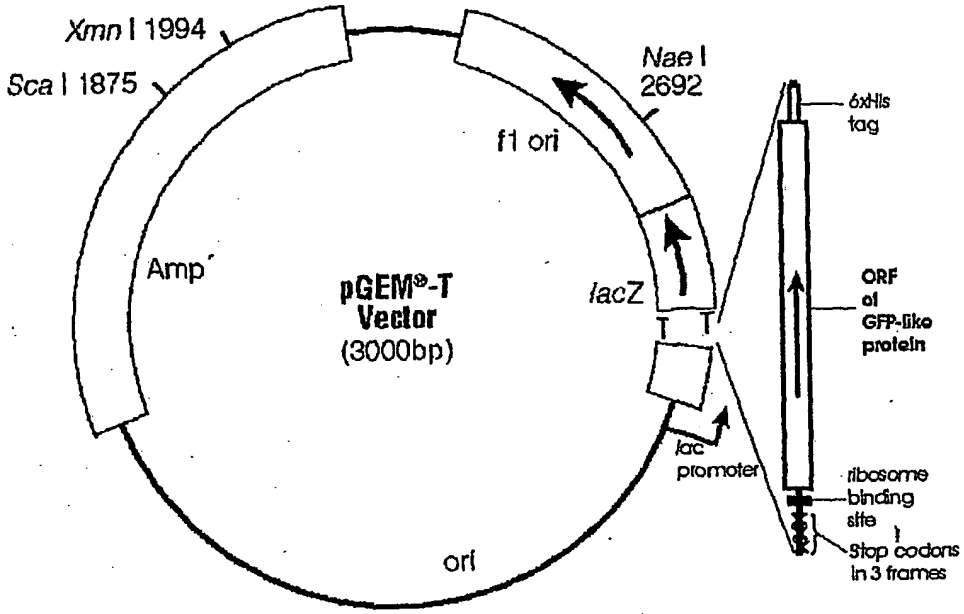

Xmn I 1994
Sca I 1875
Nae I
2692
f1 ori
Amp
pGEM®-T
Vector
(3000bp)
lacZ
T T
6xHis
tag
ORF
of
GFP-like
protein
lac
promoter
ribosome
binding
site
Stop codons
in 3 frames
ori

FIG. 1

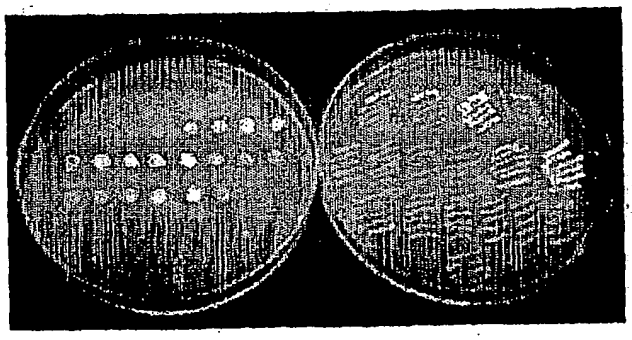


FIG. 2

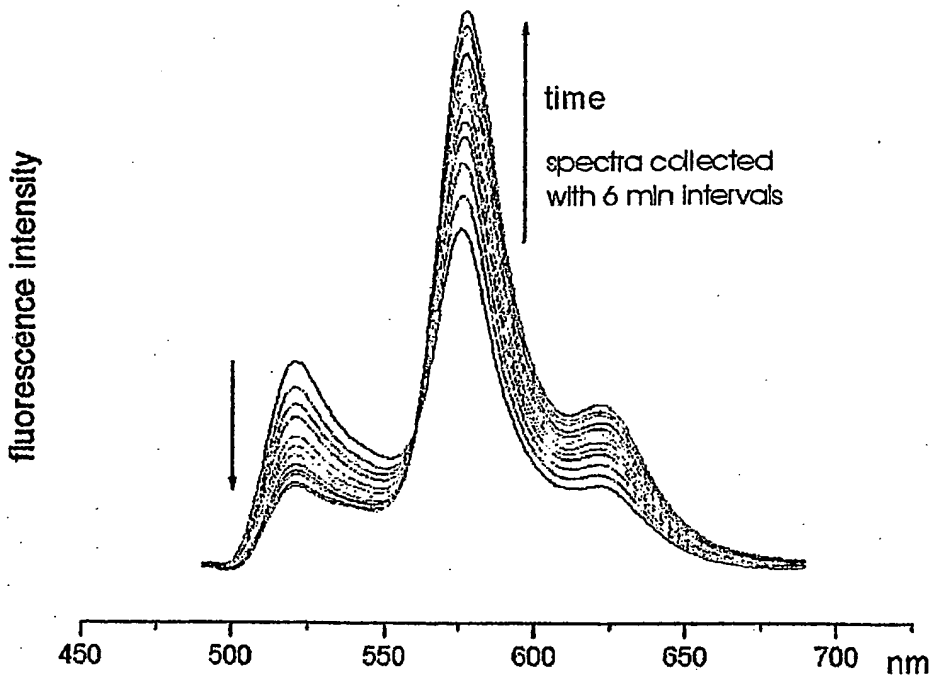

time
spectra collected
with 6 min intervals
fluorescence intensity
450
500
550
600
650
700
nm

FIG. 3A

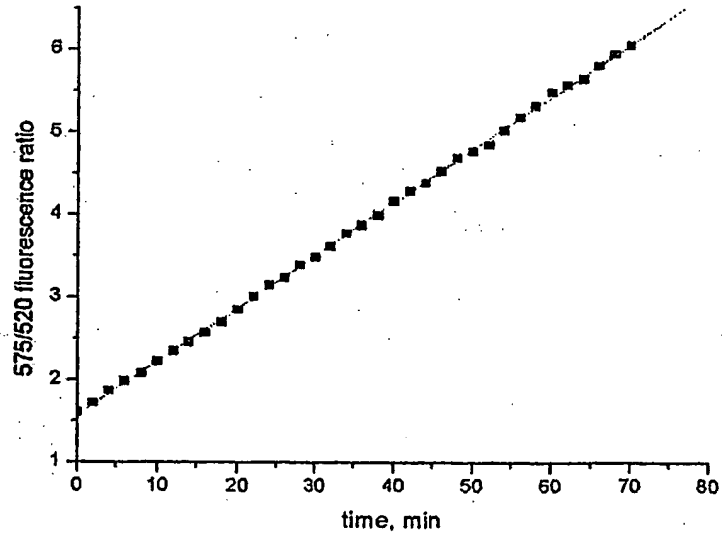

575/520 fluorescence ratio
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80
time, min

FIG. 3B

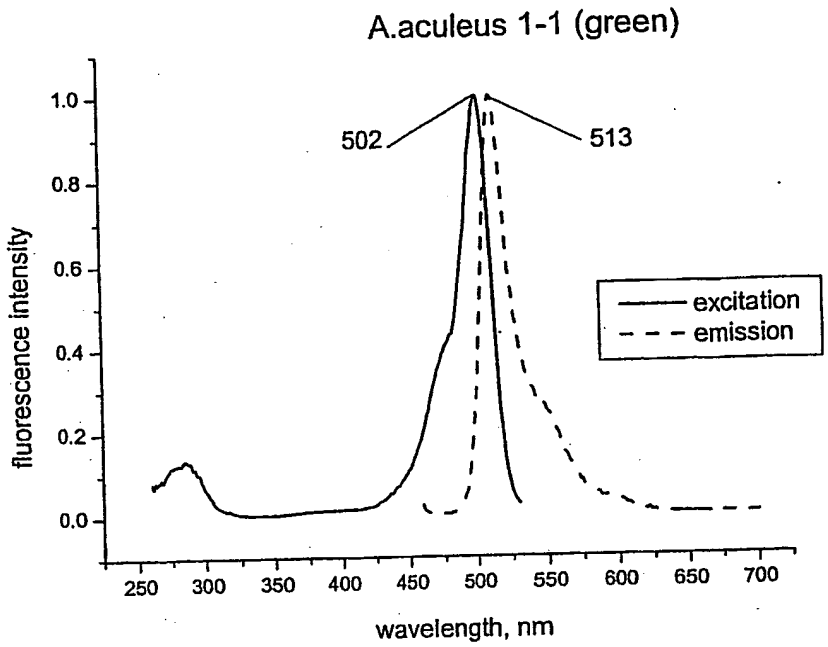

A.aculeus 1-1 (green)
502
513
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 4

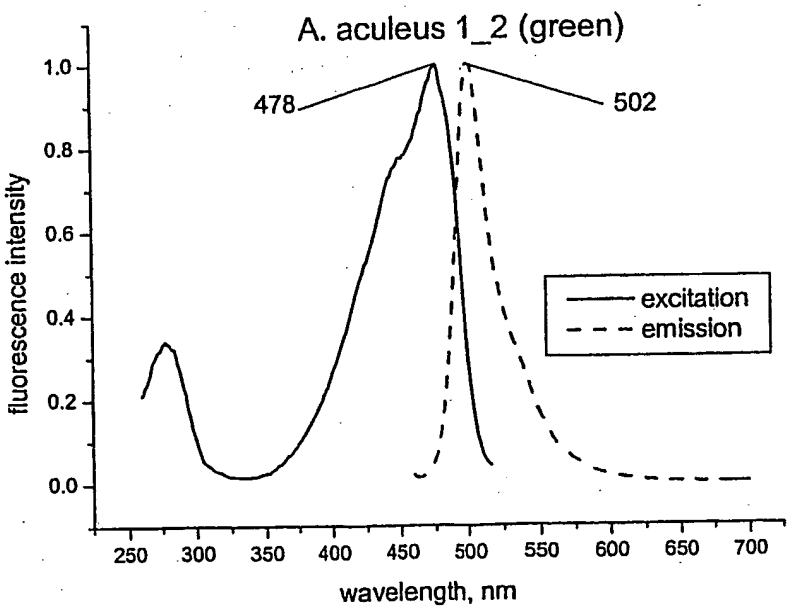

A. aculeus 1_2 (green)
478
502
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 5

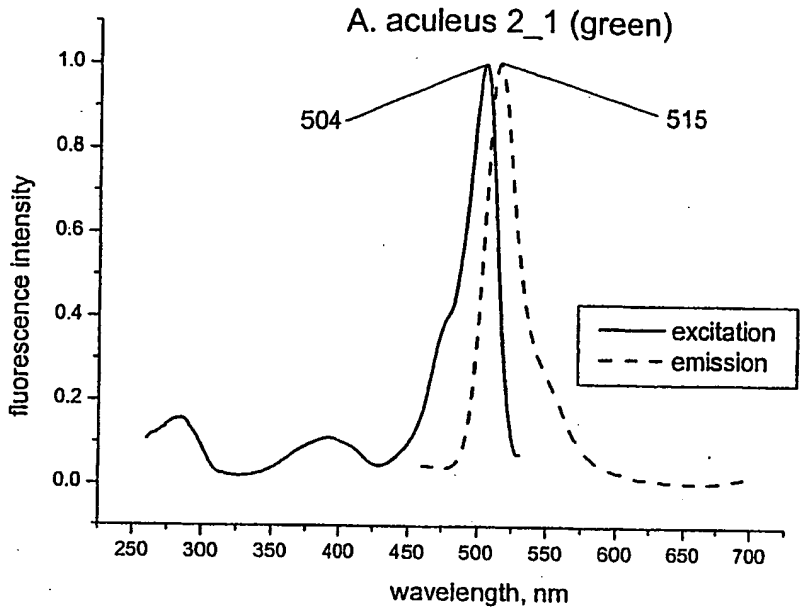

A. aculeus 2_1 (green)
504
515
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 6

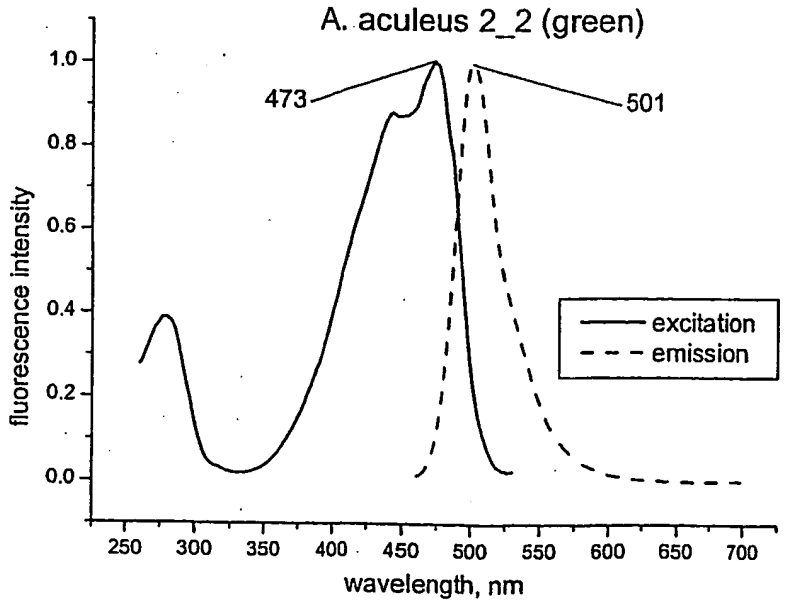

A. aculeus 2_2 (green)
473
501
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 7

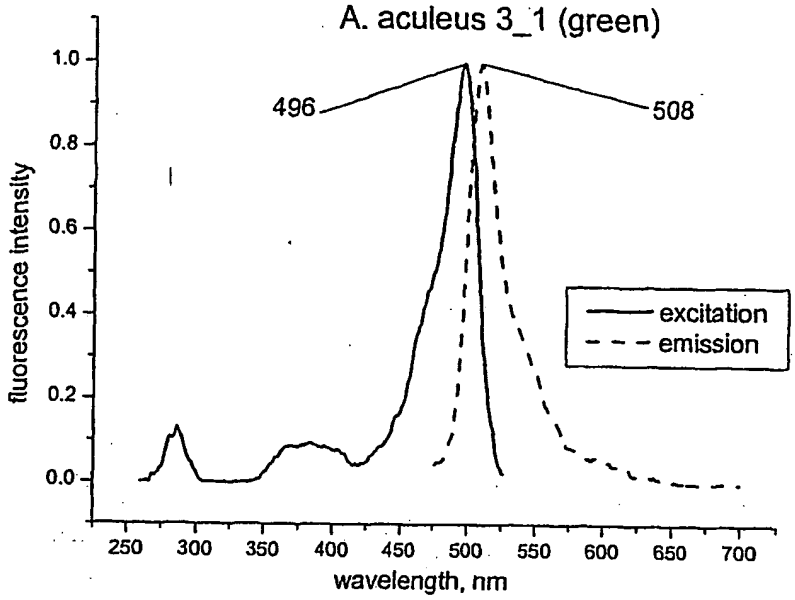

A. aculeus 3_1 (green)
496
508
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 8

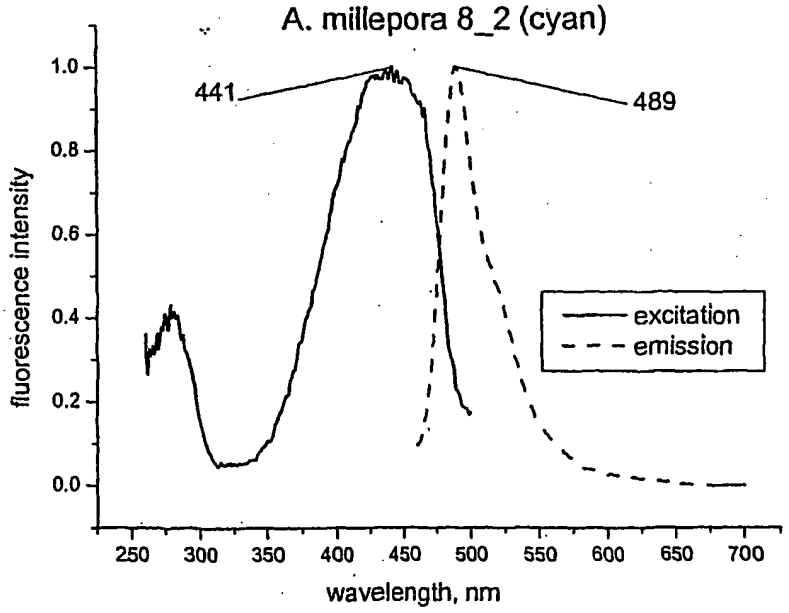

A. millepora 8_2 (cyan)
441
489
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 9

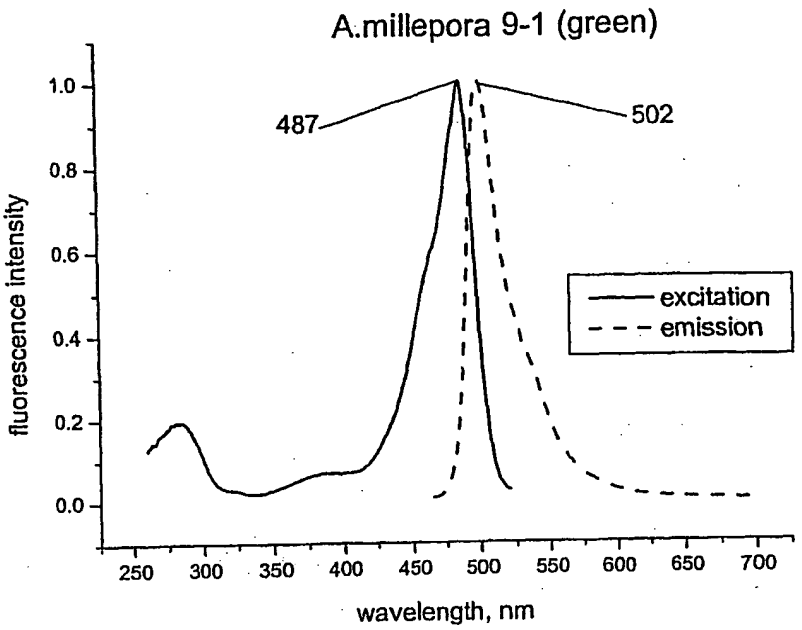

A.millepora 9-1 (green)
487
502
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 10

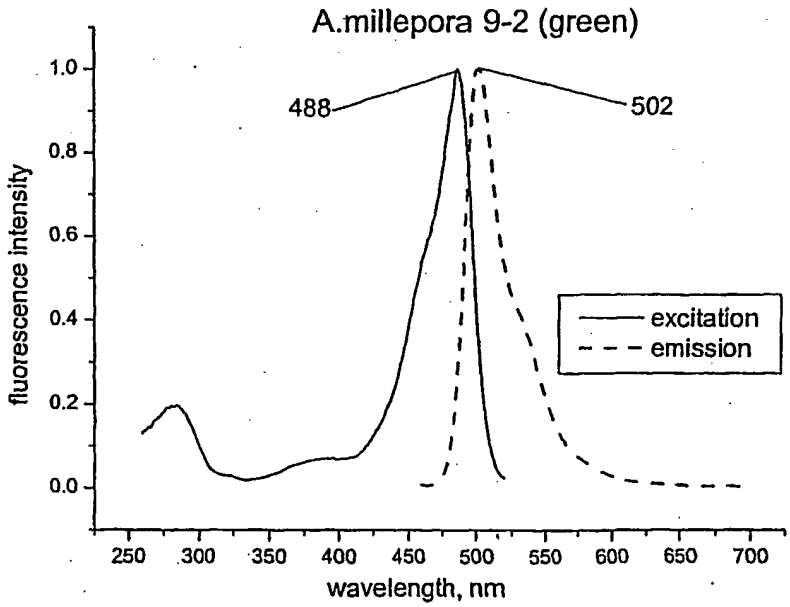

A.millepora 9-2 (green)
488
502
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 11

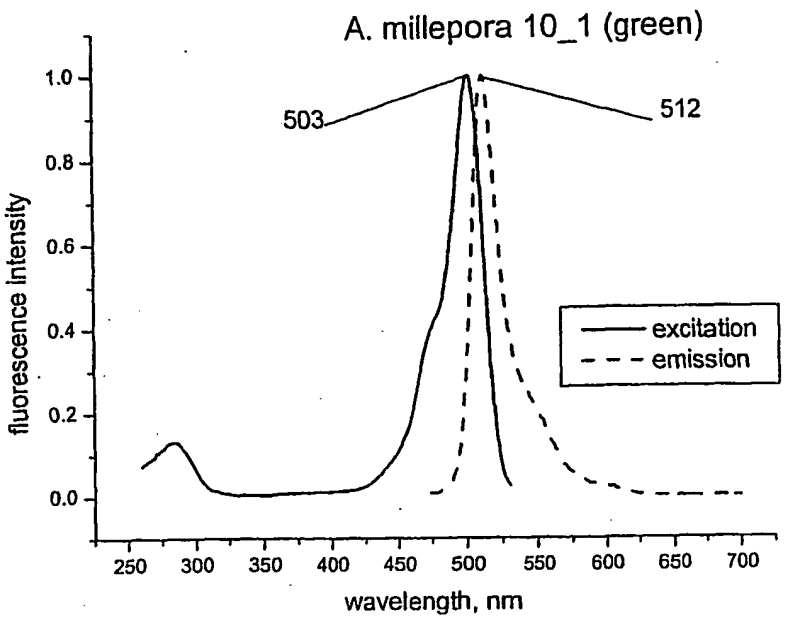

A. millepora 10_1 (green)
503
512
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 12

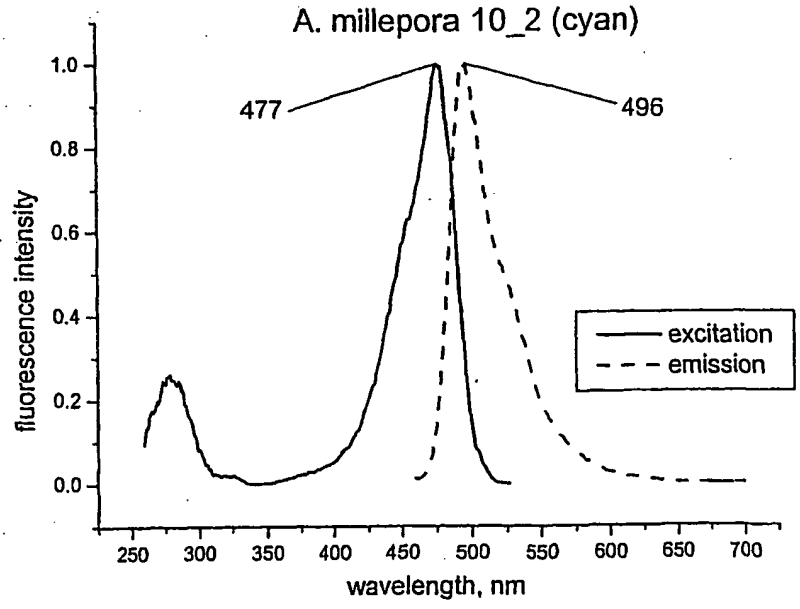

A. millepora 10_2 (cyan)
477
496
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 13

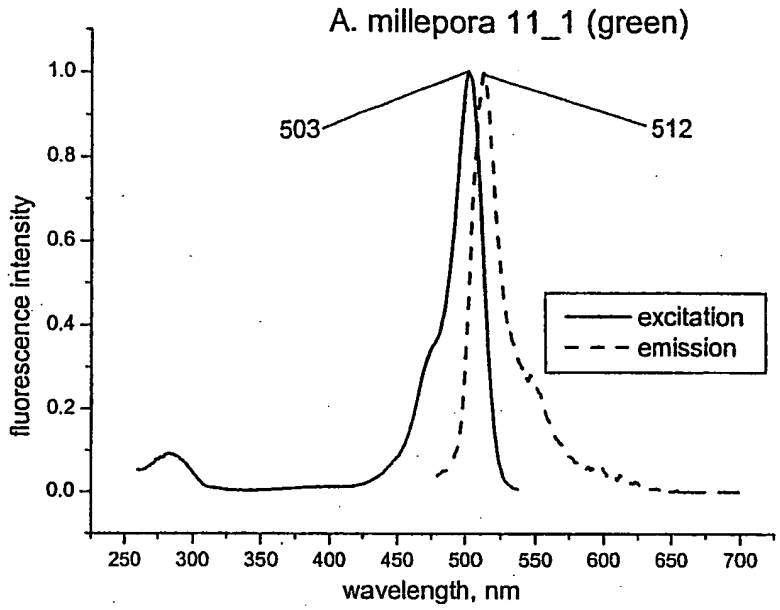

A. millepora 11_1 (green)
503
512
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 14

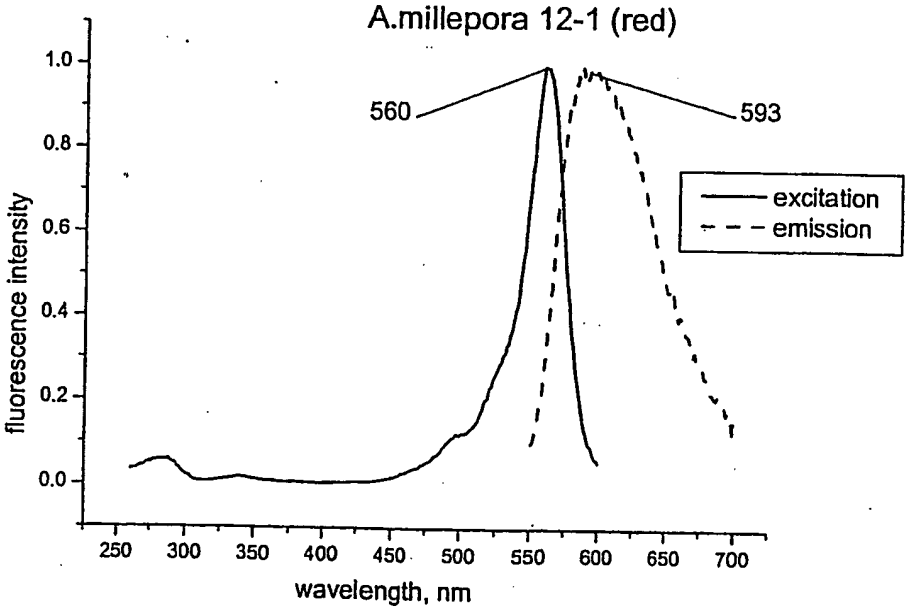

A.millepora 12-1 (red)
560
593
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 15

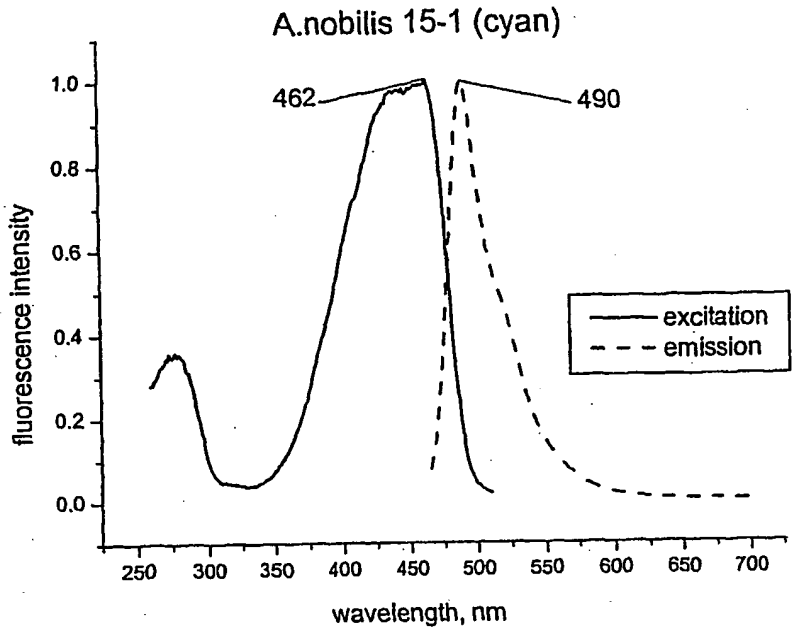

A.nobilis 15-1 (cyan)
462
490
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 16

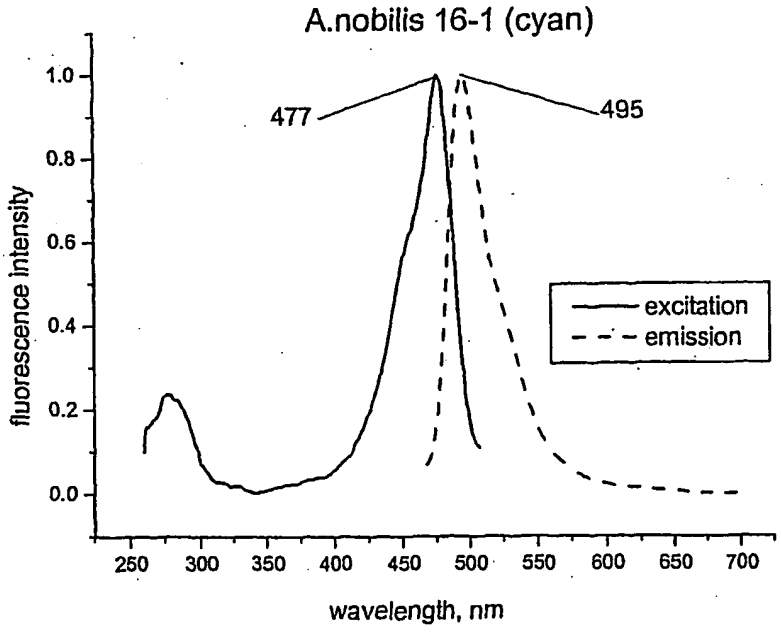

A.nobilis 16-1 (cyan)
477
495
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 17

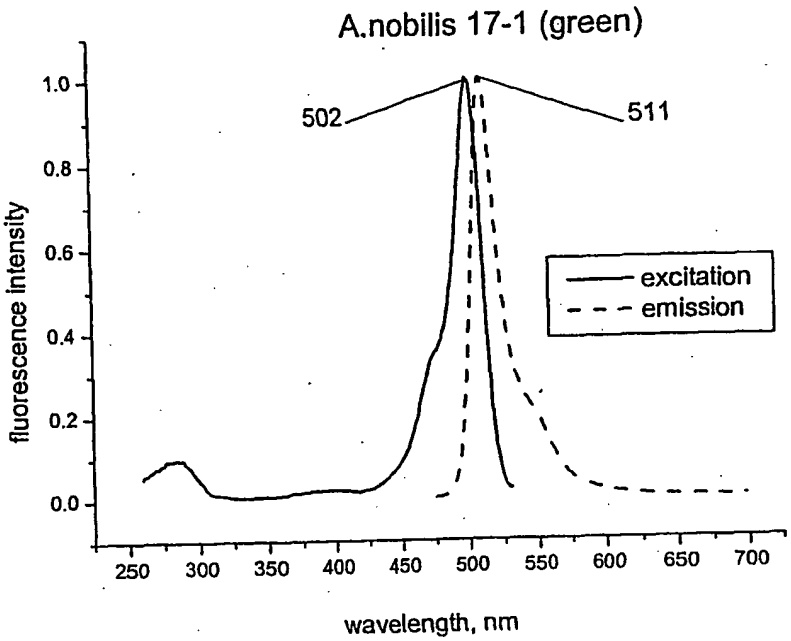

A.nobilis 17-1 (green)
502
511
excitation
emission
fluorescence intensity
wavelength, nm

FIG. 18

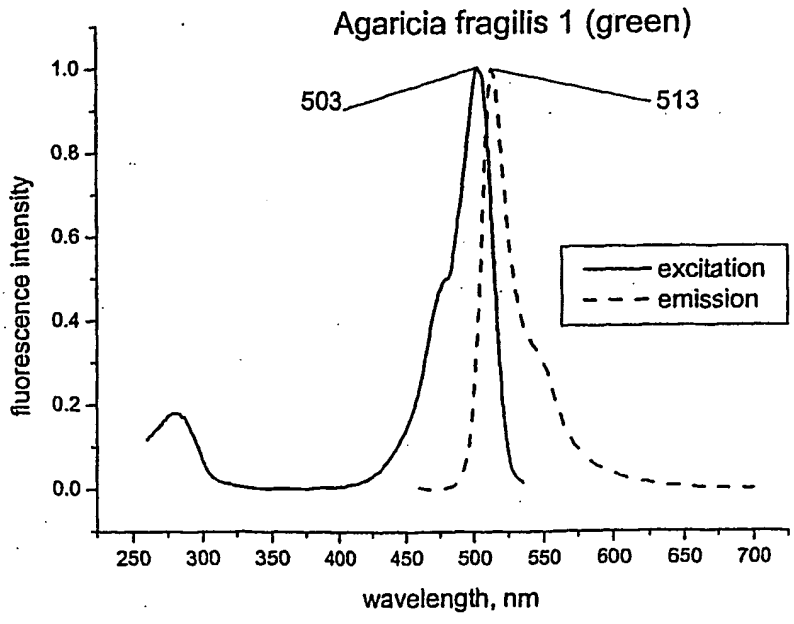

Agaricia fragilis 1 (green)
503
513
excitation
emission
fluorescence intensity
wavelength, nm

FIG. 19

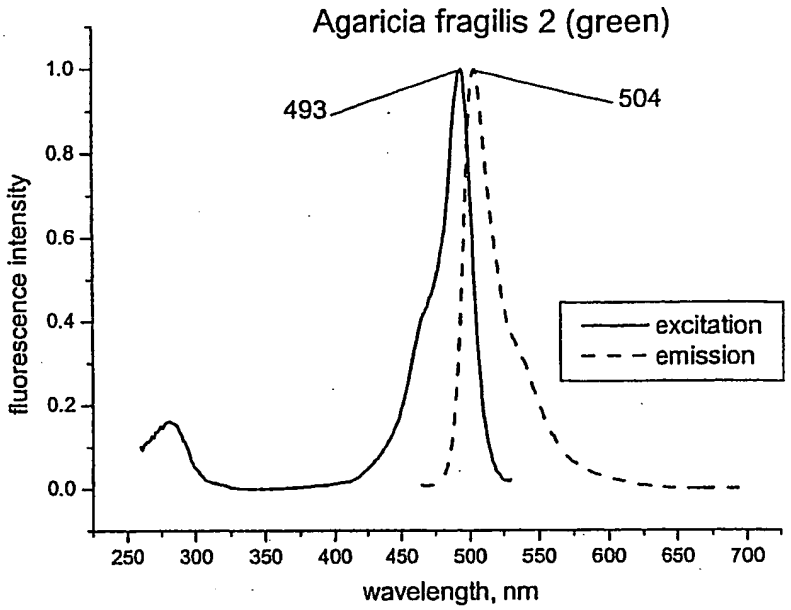

Agaricia fragilis 2 (green)
493
504
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 20

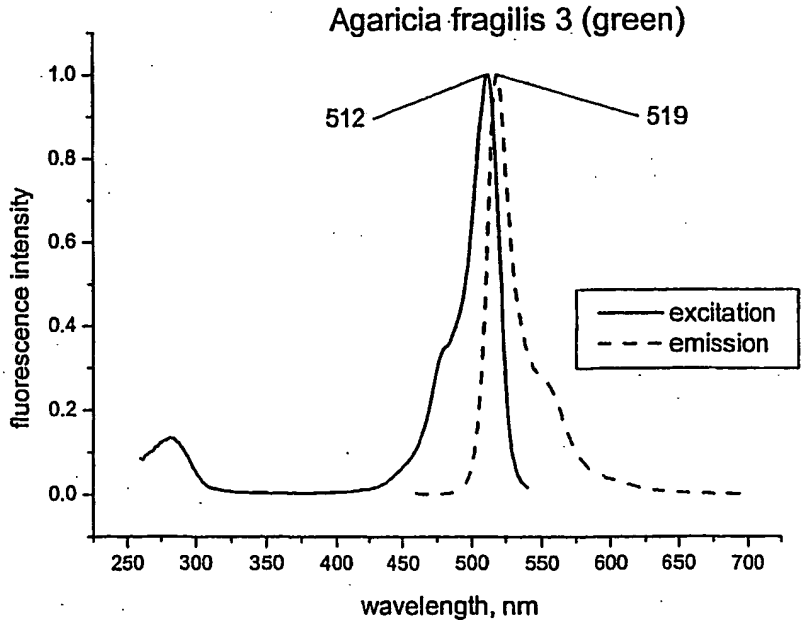

Agaricia fragilis 3 (green)
512
519
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 21

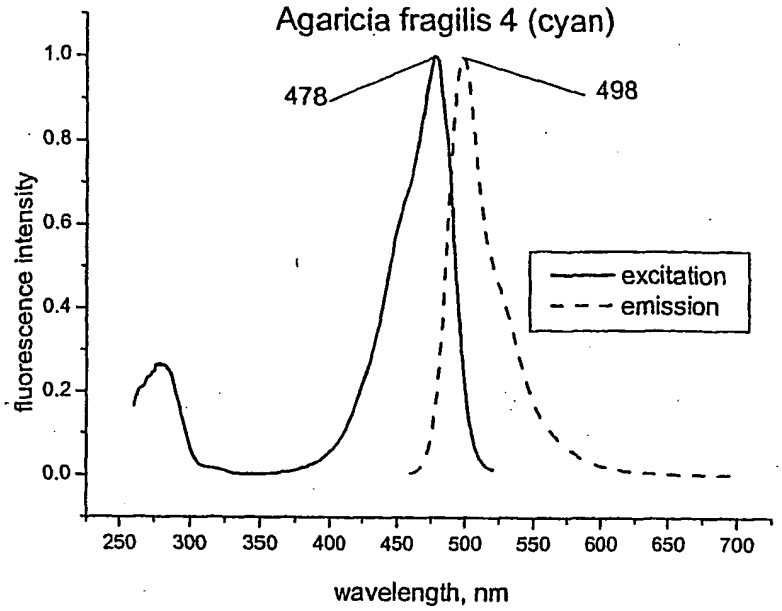

Agaricia fragilis 4 (cyan)
478
498
excitation
emission
fluorescence intensity
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700
wavelength, nm

FIG. 22

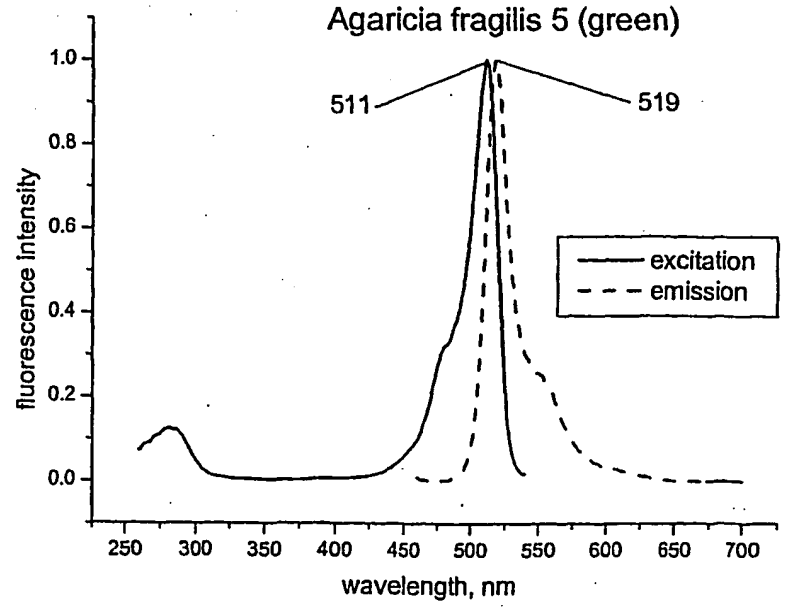

Agaricia fragilis 5 (green)
511
519
excitation
emission
fluorescence intensity
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700
wavelength, nm

FIG. 23

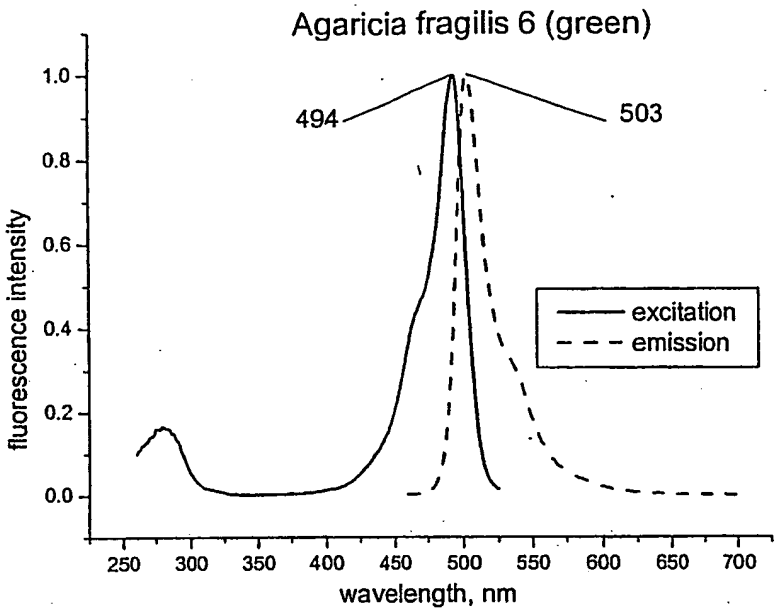

Agaricia fragilis 6 (green)
494
503
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 24

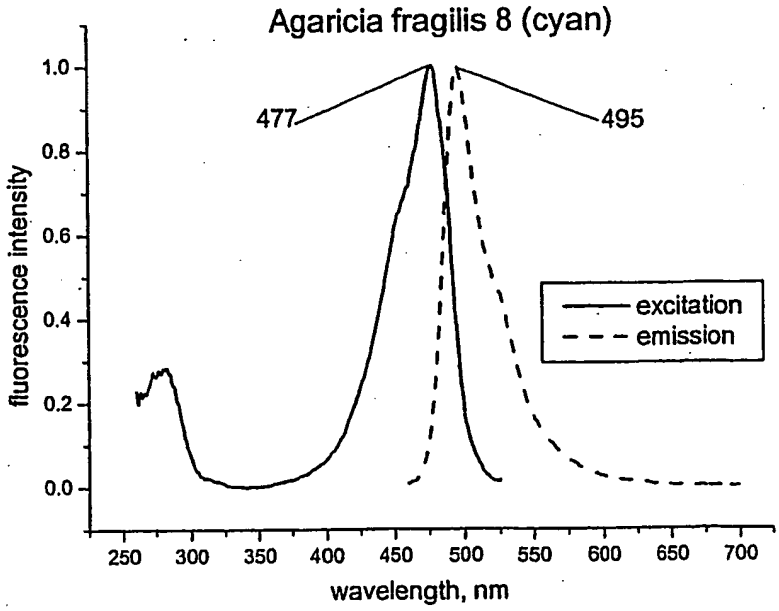

Agaricia fragilis 8 (cyan)
477
495
excitation
emission
fluorescence intensity
wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
550
600
650
700

FIG. 25

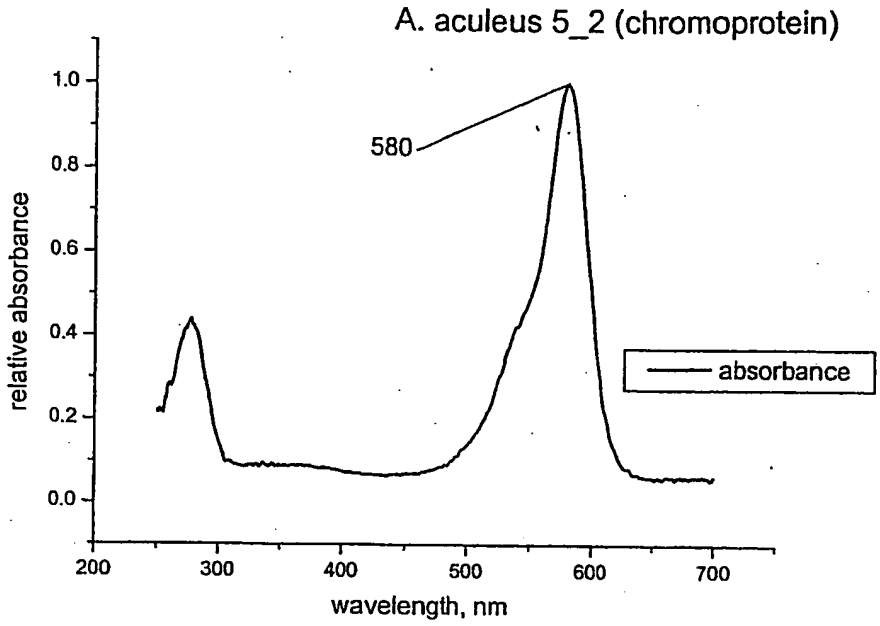

A. aculeus 5_2 (chromoprotein)
580
relative absorbance
1.0
0.8
0.6
0.4
0.2
0.0
200
300
400
500
600
700
wavelength, nm
absorbance

FIG. 26

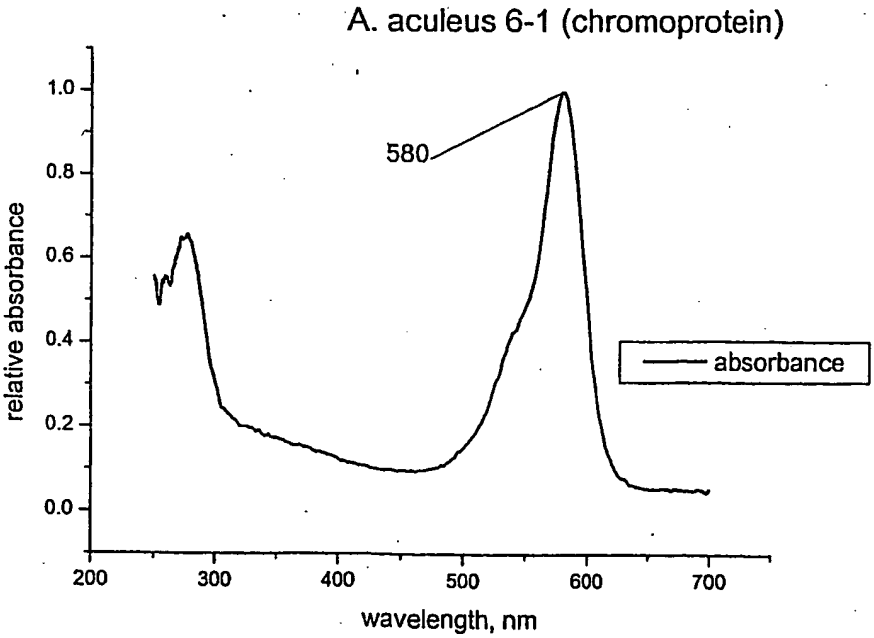

A. aculeus 6-1 (chromoprotein)
580
relative absorbance
1.0
0.8
0.6
0.4
0.2
0.0
200
300
400
500
600
700
wavelength, nm
absorbance

FIG. 27

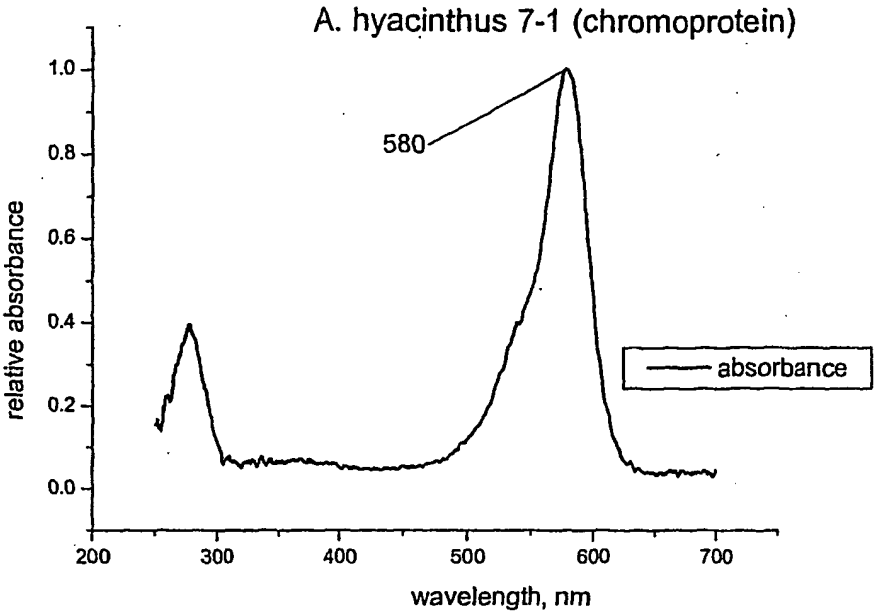

A. hyacinthus 7-1 (chromoprotein)
580
relative absorbance
1.0
0.8
0.6
0.4
0.2
0.0
200
300
400
500
600
700
wavelength, nm
absorbance

FIG. 28

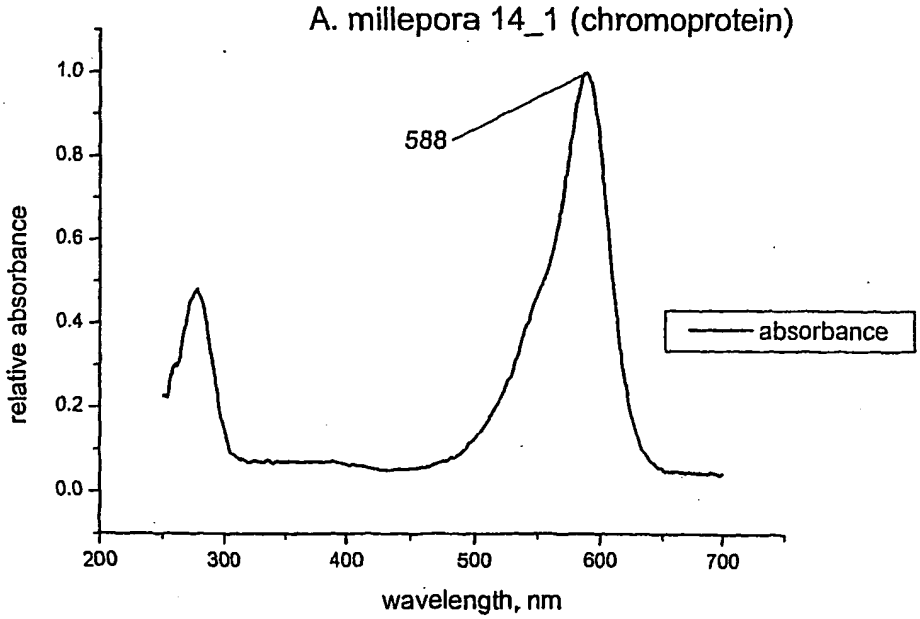

A. millepora 14_1 (chromoprotein)
588
relative absorbance
1.0
0.8
0.6
0.4
0.2
0.0
200
300
400
500
600
700
wavelength, nm
absorbance

FIG. 29

SEQUENCE LISTING

<110> University of Florida Research Foundation, Inc.
Matz, Mikhail
Kelmanson, Ilya
Salih, Anya
Meleshkevitch, Ella

<120> Novel Fluorescent and Colored Proteins, and Polynucleotides that Encode These Proteins

<130> UF-364XC1

<150> US 60/472,196
<151> 2003-05-20

<160> 96

<170> PatentIn version 3.2

<210> 1
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' heel of upstream primer

<400> 1
ttgattgatt gaaggagaaa tatcatg 27

<210> 2
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' heel of downstream primer

<400> 2
ttattagtga tggtgatggt gatg 24

<210> 3
<211> 675
<212> DNA
<213> Montastraea cavernosa

<400> 3
atgagtgtga ttaagtcaga catgaagatc aagctgccta tggaaggcac tgtaaacggg 60

cacaagtttg tcatcacagg agaaggagaa ggcaagcctt tccagggaac acacactata 120

acccttaaag tcaaagaagg gggacctctg cctttccctt acgacatctt gacaacagca 180

ttccagtacg gcaacagggt attcaccaaa tacccaagag acataccaga ctatttcaag 240

cagtcgtttc ctgaggggta ttcctgggaa agaagcatga ctttcgaaga ccagggcatt 300

tgcaccgtca caagcgacat aaagttggaa ggcgactgtt ttttctacga aattcgattt 360

tatggtgtga actttccctc caatggtcca gttatgcaga agaagacgct gaaatgggag 420

ccatccactg agaatatgta cgtgcgtgat ggagtgctac tgggggatgt taacaggact 480

ctgttgcttg aaggagataa acatcaccga tgtaacttca gaagtactta cagggcgaag 540

aagggtgtcg tgttgccaga atatcacttt gtggaccacc gaattgaaat tctgagccat 600

gacaaagatt acaacaccgt tgaggtgtat gagaatgccg ttgctcgccc ttctatgctg 660

ccgagtaagg cctaa 675

<210> 4
<211> 678
<212> DNA
<213> Montastraea cavernosa

<400> 4
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cacaacttcg tgattgaagg agaaggaaaa ggcaagcctt tcgagggaac acagactata 120

aaccttacag tcaaagaagg cggacctctg ccttttgctt acgatatctt gacagcagca 180

ttccagtacg gcaacagggc attcaccaaa tacccaagag acatagcaga ctatttcaag 240

cagtcttttc ctgaggggta ttcctgggaa cgaagcatga cttatgaaga ccagggcatt 300

tgcatcatca agagcgacat aagaatggaa ggcgactgct ttatctatga aattcgatat 360

gatggtgtga actttccccc aagtggtcca gttatgcaaa agaagacgct gaaatgggag 420

ccatccactg agaaaatgta tgtgcgtgat ggagtgctga agggtgatgt taacatggct 480

ctgttgcttg aaggaggtgg ccattaccga tgtgactttc gaagtactta caaagcgaag 540

aaacgtgttc agttgccaga ctatcacttt gtggaccacc gcattgagat tttgagccat 600

gacaatgact acaacaccgt aaagctgtct gagaatgccg aggctcgcta ttctatgctg 660

ccgagtcagg ccaagtaa 678

<210> 5
<211> 678
<212> DNA
<213> Montastraea cavernosa

<400> 5
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cacaagttcg tgattaaagg agaaggagag ggcaagcctt tcgagggaac gcagactata 120

aaccttacag tcaaagaagg cgcacctctc ccttttgctt acgacatctt gacatcagca 180

ttccagtatg gcaacagggt attcaccaaa tatccagacg atataccaga ctatttcaag 240

cagacgtttc ctgaagggta ttcgtgggag cgaatcatgg cttatgaaga ccagagtatt 300

tgcacggcca caagcgacat aaaaatggaa ggcgactgtt ttatctacga aattcaattt 360

catggtgtga actttccacc caatggtcca gttatgcaga agaagacgct gaaatgggaa 420

ccatccaccg agaaaatgta tgtgcgtgat ggagtgctga agggtgatgt taacatggct 480

ctgttgcttg aaggaggtgg ccattaccga tgtgacttca gaagtactta caaagcgaag 540

aaggatgttc atttgccaga ctatcactac gtggaccacc gcattgagat tttgagccat 600

gacaaagatt acaaaaatgt tacgctgtat gagcatgcca aagctcgcta ttctatgctg 660

ccgagtaagg ccaagtaa 678

<210> 6
<211> 693
<212> DNA
<213> Scolymia cubensis

<400> 6
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actgtaaacg ggcactactt caagattgta ggagagggtg atggcactcc ttttgaggga 120

aaacagactt tacacctcaa ggtcaaagag ggcgcacctc tgccttttgc ctacgatatc 180

ctgacaacag ctcttcatta cggaaacagg gtattcgtcg aatacccaga aaacatccca 240

gactatttca agcagtcgtt ccctaaggga tattcatggg aaagaagcct aactttcgaa 300

gacgggggaa tttgcatcgc cagaagcgac atcaaaatgg ttggcgacac tttccataac 360

gaggttcaat tttacggggt aaactttccc cccaatggtc ctgttatgca gaggcacacg 420

gtgaaatggg agccatccac tgagaagatt tatgtgcgtg atggagtgtt gacgggtgat 480

attaccatgg ctctgttgct taaaggaggt acccattacc gatgtgactt cagaactact 540

tataaagcta aggagaaggg tcccaagttc ccaggctatc accttgtcga tcattgtatt 600

gagattacaa gccatgacaa agattacaac gtggttgagc tgtatgagca tgccgtcgct 660

cattctggat tgccggacag tgccaatcga taa 693

<210> 7
<211> 224
<212> PRT
<213> Montastraea cavernosa

<400> 7

Met Ser Val Ile Lys Ser Asp Met Lys Ile Lys Leu Pro Met Glu Gly
1 5 10 15

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Thr Val Asn Gly His Lys Phe Val Ile Thr Gly Glu Gly Glu Gly Lys
            20                  25                  30

Pro Phe Gln Gly Thr His Thr Ile Thr Leu Lys Val Lys Glu Gly Gly
        35                  40                  45

Pro Leu Pro Phe Pro Tyr Asp Ile Leu Thr Thr Ala Phe Gln Tyr Gly
    50                  55                  60

Asn Arg Val Phe Thr Lys Tyr Pro Arg Asp Ile Pro Asp Tyr Phe Lys
65                  70                  75                  80

Gln Ser Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ser Met Thr Phe Glu
                85                  90                  95

Asp Gln Gly Ile Cys Thr Val Thr Ser Asp Ile Lys Leu Glu Gly Asp
            100                 105                 110

Cys Phe Phe Tyr Glu Ile Arg Phe Tyr Gly Val Asn Phe Pro Ser Asn
        115                 120                 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu
    130                 135                 140

Asn Met Tyr Val Arg Asp Gly Val Leu Leu Gly Asp Val Asn Arg Thr
145                 150                 155                 160

Leu Leu Leu Glu Gly Asp Lys His His Arg Cys Asn Phe Arg Ser Thr
                165                 170                 175

Tyr Arg Ala Lys Lys Gly Val Val Leu Pro Glu Tyr His Phe Val Asp
            180                 185                 190

His Arg Ile Glu Ile Leu Ser His Asp Lys Asp Tyr Asn Thr Val Glu
        195                 200                 205

Val Tyr Glu Asn Ala Val Ala Arg Pro Ser Met Leu Pro Ser Lys Ala
    210                 215                 220


<210>  8
<211>  225
<212>  PRT
<213>  Montastraea cavernosa

<400>  8

Met Ser Val Ile Lys Pro Asp Met Lys Ile Lys Leu Arg Met Glu Gly
1               5                   10                  15

Ala Val Asn Gly His Asn Phe Val Ile Glu Gly Glu Gly Lys Gly Lys
            20                  25                  30

Pro Phe Glu Gly Thr Gln Thr Ile Asn Leu Thr Val Lys Glu Gly Gly
        35                  40                  45

Pro Leu Pro Phe Ala Tyr Asp Ile Leu Thr Ala Ala Phe Gln Tyr Gly
    50                  55                  60
```

```
Asn Arg Ala Phe Thr Lys Tyr Pro Arg Asp Ile Ala Asp Tyr Phe Lys
65                  70                  75                  80

Gln Ser Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ser Met Thr Tyr Glu
                85                  90                  95

Asp Gln Gly Ile Cys Ile Ile Lys Ser Asp Ile Arg Met Glu Gly Asp
            100                 105                 110

Cys Phe Ile Tyr Glu Ile Arg Tyr Asp Gly Val Asn Phe Pro Pro Ser
        115                 120                 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu
    130                 135                 140

Lys Met Tyr Val Arg Asp Gly Val Leu Lys Gly Asp Val Asn Met Ala
145                 150                 155                 160

Leu Leu Leu Glu Gly Gly Gly His Tyr Arg Cys Asp Phe Arg Ser Thr
                165                 170                 175

Tyr Lys Ala Lys Lys Arg Val Gln Leu Pro Asp Tyr His Phe Val Asp
            180                 185                 190

His Arg Ile Glu Ile Leu Ser His Asp Asn Asp Tyr Asn Thr Val Lys
        195                 200                 205

Leu Ser Glu Asn Ala Glu Ala Arg Tyr Ser Met Leu Pro Ser Gln Ala
    210                 215                 220

Lys
225
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<210> 9
<211> 225
<212> PRT
<213> Montastraea cavernosa

<400> 9

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Met Ser Val Ile Lys Pro Asp Met Lys Ile Lys Leu Arg Met Gln Gly
1               5                   10                  15

Val Val Asn Gly His Lys Phe Val Ile Lys Gly Glu Gly Glu Gly Lys
            20                  25                  30

Pro Phe Glu Gly Thr Gln Thr Ile Asn Leu Thr Val Lys Glu Gly Ala
        35                  40                  45

Pro Leu Pro Phe Ala Tyr Asp Ile Leu Thr Ser Ala Phe Gln Tyr Gly
    50                  55                  60

Asn Arg Val Phe Thr Lys Tyr Pro Asp Asp Ile Pro Asp Tyr Phe Lys
65                  70                  75                  80

Gln Thr Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ile Met Ala Tyr Glu
                85                  90                  95
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Asp Gln Ser Ile Cys Thr Ala Thr Ser Asp Ile Lys Met Glu Gly Asp
100 105 110

Cys Phe Ile Tyr Glu Ile Gln Phe His Gly Val Asn Phe Pro Pro Asn
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu
130 135 140

Lys Met Tyr Val Arg Asp Gly Val Leu Lys Gly Asp Val Asn Met Ala
145 150 155 160

Leu Leu Leu Glu Gly Gly Gly His Tyr Arg Cys Asp Phe Arg Ser Thr
165 170 175

Tyr Lys Ala Lys Lys Asp Val His Leu Pro Asp Tyr His Tyr Val Asp
180 185 190

His Arg Ile Glu Ile Leu Ser His Asp Lys Asp Tyr Lys Asn Val Thr
195 200 205

Leu Tyr Glu His Ala Lys Ala Arg Tyr Ser Met Leu Pro Ser Lys Ala
210 215 220

Lys
225

<210> 10
<211> 230
<212> PRT
<213> Scolymia cubensis

<400> 10

Met Ser Ala Ile Lys Thr Val Val Lys Gln Phe Met Lys Ile Lys Met
1 5 10 15

Ser Leu Glu Gly Thr Val Asn Gly His Tyr Phe Lys Ile Val Gly Glu
20 25 30

Gly Asp Gly Thr Pro Phe Glu Gly Lys Gln Thr Leu His Leu Lys Val
35 40 45

Lys Glu Gly Ala Pro Leu Pro Phe Ala Tyr Asp Ile Leu Thr Thr Ala
50 55 60

Leu His Tyr Gly Asn Arg Val Phe Val Glu Tyr Pro Glu Asn Ile Pro
65 70 75 80

Asp Tyr Phe Lys Gln Ser Phe Pro Lys Gly Tyr Ser Trp Glu Arg Ser
85 90 95

Leu Thr Phe Glu Asp Gly Gly Ile Cys Ile Ala Arg Ser Asp Ile Lys
100 105 110

Met Val Gly Asp Thr Phe His Asn Glu Val Gln Phe Tyr Gly Val Asn
115 120 125

Phe Pro Pro Asn Gly Pro Val Met Gln Arg His Thr Val Lys Trp Glu
130 135 140

Pro Ser Thr Glu Lys Ile Tyr Val Arg Asp Gly Val Leu Thr Gly Asp
145 150 155 160

Ile Thr Met Ala Leu Leu Leu Lys Gly Gly Thr His Tyr Arg Cys Asp
165 170 175

Phe Arg Thr Thr Tyr Lys Ala Lys Glu Lys Gly Pro Lys Phe Pro Gly
180 185 190

Tyr His Leu Val Asp His Cys Ile Glu Ile Thr Ser His Asp Lys Asp
195 200 205

Tyr Asn Val Val Glu Leu Tyr Glu His Ala Val Ala His Ser Gly Leu
210 215 220

Pro Asp Ser Ala Asn Arg
225 230

<210> 11
<211> 726
<212> DNA
<213> Montastraea cavernosa

<400> 11
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cctatggaag gcactgtaaa cgggcacaag tttgtcatca caggagaagg agaaggcaag 120

cctttccagg gaacacacac tataaccctt aaagtcaaag aaggggacc tctgcctttc 180

ccttacgaca tcttgacaac agcattccag tacggcaaca gggtattcac caaataccca 240

agagacatac cagactattt caagcagtcg tttcctgagg ggtattcctg ggaaagaagc 300

atgactttcg aagaccaggg catttgcacc gtcacaagcg acataaagtt ggaaggcgac 360

tgtttttttct acgaaattcg attttatggt gtgaactttc cctccaatgg tccagttatg 420

cagaagaaga cgctgaaatg ggagccatcc actgagaata tgtacgtgcg tgatggagtg 480

ctactggggg atgttaacag gactctgttg cttgaaggag ataaacatca ccgatgtaac 540

ttcagaagta cttacagggc gaagaagggt gtcgtgttgc cagaatatca ctttgtggac 600

caccgaattg aaattctgag ccatgacaaa gattacaaca ccgttgaggt gtatgagaat 660

gccgttgctc gcccttctat gctgccgagt aaggccgaaa gtgcacatca ccatcaccat 720

cactaa 726

<210> 12
<211> 729
<212> DNA

<213> Montastraea cavernosa

<400> 12
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cgtatggaag gcgctgtaaa cgggcacaac ttcgtgattg aaggagaagg aaaaggcaag 120

cctttcgagg gaacacagac tataaacctt acagtcaaag aaggcggacc tctgcctttt 180

gcttacgata tcttgacagc agcattccag tacggcaaca gggcattcac caaataccca 240

agagacatag cagactattt caagcagtct tttcctgagg ggtattcctg ggaacgaagc 300

atgacttatg aagaccaggg catttgcatc atcaagagcg acataagaat ggaaggcgac 360

tgctttatct atgaaattcg atatgatggt gtgaactttc ccccaagtgg tccagttatg 420

caaaagaaga cgctgaaatg ggagccatcc actgagaaaa tgtatgtgcg tgatggagtg 480

ctgaagggtg atgttaacat ggctctgttg cttgaaggag gtggccatta ccgatgtgac 540

tttcgaagta cttacaaagc gaagaaacgt gttcagttgc cagactatca ctttgtggac 600

caccgcattg agattttgag ccatgacaat gactacaaca ccgtaaagct gtctgagaat 660

gccgaggctc gctattctat gctgccgagt caggccaagg aaagtgcaca tcaccatcac 720

catcactaa 729

<210> 13
<211> 729
<212> DNA
<213> Montastraea cavernosa

<400> 13
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cgtatgcaag gcgttgtaaa cgggcacaag ttcgtgatta aaggagaagg agagggcaag 120

cctttcgagg gaacgcagac tataaacctt acagtcaaag aaggcgcacc tctccctttt 180

gcttacgaca tcttgacatc agcattccag tatggcaaca gggtattcac caaatatcca 240

gacgatatac cagactattt caagcagacg tttcctgaag ggtattcgtg ggagcgaatc 300

atggcttatg aagaccagag tatttgcacg gccacaagcg acataaaaat ggaaggcgac 360

tgttttatct acgaaattca atttcatggt gtgaactttc cacccaatgg tccagttatg 420

cagaagaaga cgctgaaatg ggaaccatcc accgagaaaa tgtatgtgcg tgatggagtg 480

ctgaagggtg atgttaacat ggctctgttg cttgaaggag gtggccatta ccgatgtgac 540

ttcagaagta cttacaaagc gaagaaggat gttcatttgc cagactatca ctacgtggac 600

caccgcattg agattttgag ccatgacaaa gattacaaaa atgttacgct gtatgagcat 660

gccaaagctc gctattctat gctgccgagt aaggccaagg aaagtgcaca tcaccatcac 720

catcactaa 729

<210> 14
<211> 741
<212> DNA
<213> Scolymia cubensis

<400> 14
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aagatcaaga tgtctttgga aggcactgta aacgggcact acttcaagat tgtaggagag 120

ggtgatggca ctccttttga gggaaaacag actttacacc tcaaggtcaa agagggcgca 180

cctctgcctt ttgcctacga tatcctgaca acagctcttc attacggaaa cagggtattc 240

gtcgaatacc cagaaaacat cccagactat ttcaagcagt cgttccctaa gggatattca 300

tgggaaagaa gcctaacttt cgaagacggg ggaatttgca tcgccagaag cgacatcaaa 360

atggttggcg acactttcca taacgaggtt caattttacg gggtaaactt tccccccaat 420

ggtcctgtta tgcagaggca cacggtgaaa tgggagccat ccactgagaa gatttatgtg 480

cgtgatggag tgttgacggg tgatattacc atggctctgt tgcttaaagg aggtacccat 540

taccgatgtg acttcagaac tacttataaa gctaaggaga agggtcccaa gttcccaggc 600

tatcaccttg tcgatcattg tattgagatt acaagccatg acaaagatta caacgtggtt 660

gagctgtatg agcatgccgt cgctcattct ggattgccgg acagtgccaa tcgattgatt 720

gattgaagga gaaatatcta a 741

<210> 15
<211> 234
<212> PRT
<213> Montastraea cavernosa

<400> 15

Met Ser Val Ile Lys Ser Asp Met Lys Ile Lys Leu Pro Met Glu Gly
1 5 10 15

Thr Val Asn Gly His Lys Phe Val Ile Thr Gly Glu Gly Glu Gly Lys
20 25 30

Pro Phe Gln Gly Thr His Thr Ile Thr Leu Lys Val Lys Glu Gly Gly
35 40 45

Pro Leu Pro Phe Pro Tyr Asp Ile Leu Thr Thr Ala Phe Gln Tyr Gly
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Arg Asp Ile Pro Asp Tyr Phe Lys
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ser Met Thr Phe Glu
85 90 95

Asp Gln Gly Ile Cys Thr Val Thr Ser Asp Ile Lys Leu Glu Gly Asp
100 105 110

Cys Phe Phe Tyr Glu Ile Arg Phe Tyr Gly Val Asn Phe Pro Ser Asn
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu
130 135 140

Asn Met Tyr Val Arg Asp Gly Val Leu Leu Gly Asp Val Asn Arg Thr
145 150 155 160

Leu Leu Leu Glu Gly Asp Lys His His Arg Cys Asn Phe Arg Ser Thr
165 170 175

Tyr Arg Ala Lys Lys Gly Val Val Leu Pro Glu Tyr His Phe Val Asp
180 185 190

His Arg Ile Glu Ile Leu Ser His Asp Lys Asp Tyr Asn Thr Val Glu
195 200 205

Val Tyr Glu Asn Ala Val Ala Arg Pro Ser Met Leu Pro Ser Lys Ala
210 215 220

Lys Glu Ser Ala His His His His His His
225 230

<210> 16
<211> 234
<212> PRT
<213> Montastraea cavernosa

<400> 16

Met Ser Val Ile Lys Pro Asp Met Lys Ile Lys Leu Arg Met Glu Gly
1 5 10 15

Ala Val Asn Gly His Asn Phe Val Ile Glu Gly Glu Gly Lys Gly Lys
20 25 30

Pro Phe Glu Gly Thr Gln Thr Ile Asn Leu Thr Val Lys Glu Gly Gly
35 40 45

Pro Leu Pro Phe Ala Tyr Asp Ile Leu Thr Ala Ala Phe Gln Tyr Gly
50 55 60

Asn Arg Ala Phe Thr Lys Tyr Pro Arg Asp Ile Ala Asp Tyr Phe Lys
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ser Met Thr Tyr Glu
85 90 95

Asp Gln Gly Ile Cys Ile Ile Lys Ser Asp Ile Arg Met Glu Gly Asp
100 105 110

```
Cys Phe Ile Tyr Glu Ile Arg Tyr Asp Gly Val Asn Phe Pro Pro Ser
        115                 120                 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu
    130                 135                 140

Lys Met Tyr Val Arg Asp Gly Val Leu Lys Gly Asp Val Asn Met Ala
145                 150                 155                 160

Leu Leu Leu Glu Gly Gly Gly His Tyr Arg Cys Asp Phe Arg Ser Thr
                165                 170                 175

Tyr Lys Ala Lys Lys Arg Val Gln Leu Pro Asp Tyr His Phe Val Asp
            180                 185                 190

His Arg Ile Glu Ile Leu Ser His Asp Asn Asp Tyr Asn Thr Val Lys
        195                 200                 205

Leu Ser Glu Asn Ala Glu Ala Arg Tyr Ser Met Leu Pro Ser Gln Ala
    210                 215                 220

Lys Glu Ser Ala His His His His His His
225                 230
```

<210> 17
<211> 234
<212> PRT
<213> Montastraea cavernosa

<400> 17

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Met Ser Val Ile Lys Pro Asp Met Lys Ile Lys Leu Arg Met Gln Gly
1               5                   10                  15

Val Val Asn Gly His Lys Phe Val Ile Lys Gly Glu Gly Glu Gly Lys
            20                  25                  30

Pro Phe Glu Gly Thr Gln Thr Ile Asn Leu Thr Val Lys Glu Gly Ala
        35                  40                  45

Pro Leu Pro Phe Ala Tyr Asp Ile Leu Thr Ser Ala Phe Gln Tyr Gly
    50                  55                  60

Asn Arg Val Phe Thr Lys Tyr Pro Asp Asp Ile Pro Asp Tyr Phe Lys
65                  70                  75                  80

Gln Thr Phe Pro Glu Gly Tyr Ser Trp Glu Arg Ile Met Ala Tyr Glu
                85                  90                  95

Asp Gln Ser Ile Cys Thr Ala Thr Ser Asp Ile Lys Met Glu Gly Asp
            100                 105                 110

Cys Phe Ile Tyr Glu Ile Gln Phe His Gly Val Asn Phe Pro Pro Asn
        115                 120                 125

Gly Pro Val Met Gln Lys Lys Thr Leu Lys Trp Glu Pro Ser Thr Glu
    130                 135                 140
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```
Lys Met Tyr Val Arg Asp Gly Val Leu Lys Gly Asp Val Asn Met Ala
145                 150                 155                 160

Leu Leu Leu Glu Gly Gly Gly His Tyr Arg Cys Asp Phe Arg Ser Thr
                165                 170                 175

Tyr Lys Ala Lys Lys Asp Val His Leu Pro Asp Tyr His Tyr Val Asp
            180                 185                 190

His Arg Ile Glu Ile Leu Ser His Asp Lys Asp Tyr Lys Asn Val Thr
        195                 200                 205

Leu Tyr Glu His Ala Lys Ala Arg Tyr Ser Met Leu Pro Ser Lys Ala
    210                 215                 220

Lys Glu Ser Ala His His His His His His
225                 230
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<210> 18
<211> 238
<212> PRT
<213> Scolymia cubensis

<400> 18

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Met Ser Ala Ile Lys Thr Val Val Lys Gln Phe Met Lys Ile Lys Met
1               5                   10                  15

Ser Leu Glu Gly Thr Val Asn Gly His Tyr Phe Lys Ile Val Gly Glu
            20                  25                  30

Gly Asp Gly Thr Pro Phe Glu Gly Lys Gln Thr Leu His Leu Lys Val
        35                  40                  45

Lys Glu Gly Ala Pro Leu Pro Phe Ala Tyr Asp Ile Leu Thr Thr Ala
    50                  55                  60

Leu His Tyr Gly Asn Arg Val Phe Val Glu Tyr Pro Glu Asn Ile Pro
65                  70                  75                  80

Asp Tyr Phe Lys Gln Ser Phe Pro Lys Gly Tyr Ser Trp Glu Arg Ser
                85                  90                  95

Leu Thr Phe Glu Asp Gly Gly Ile Cys Ile Ala Arg Ser Asp Ile Lys
            100                 105                 110

Met Val Gly Asp Thr Phe His Asn Glu Val Gln Phe Tyr Gly Val Asn
        115                 120                 125

Phe Pro Pro Asn Gly Pro Val Met Gln Arg His Thr Val Lys Trp Glu
    130                 135                 140

Pro Ser Thr Glu Lys Ile Tyr Val Arg Asp Gly Val Leu Thr Gly Asp
145                 150                 155                 160

Ile Thr Met Ala Leu Leu Leu Lys Gly Gly Thr His Tyr Arg Cys Asp
                165                 170                 175
```

Phe Arg Thr Thr Tyr Lys Ala Lys Glu Lys Gly Pro Lys Phe Pro Gly
180 185 190

Tyr His Leu Val Asp His Cys Ile Glu Ile Thr Ser His Asp Lys Asp
195 200 205

Tyr Asn Val Val Glu Leu Tyr Glu His Ala Val Ala His Ser Gly Leu
210 215 220

Pro Asp Ser Ala Asn Arg Gln Ser His His His His His His
225 230 235

<210> 19
<211> 765
<212> DNA
<213> Acropora aculeus

<400> 19
ttgattgatt gaaggagaaa tatcatgatc aagccatcta tcctcaacat gtcttattca 60

aagcagggca tcgtacaaga aatgaagacg aaataccgta tggaaggcag tgtcaatggc 120

catgaattca cgatcgaagg tgtaggaact gggtaccctt acgaagggaa acagatgtcc 180

gaattagtga tcatcaagcc taagggaaag ccccttccat tctcctttga catactgtca 240

tcagtctttc aatatggaaa caggtgcttc acaaagtacc ctgcagacat gcctgactat 300

ttcaagcaag cattcccaga tggaatgtca tatgaaaggt catttctatt tgaggatgga 360

gcagttgcta cagccagctg gaacattcgt ctcgaaggaa attgcttcat ccacaattcc 420

atctttcatg gcgtaaactt tcccgatgat ggacccgtaa tgaaaaagaa gacaattggc 480

tgggataagt ccttcgaaaa aatgactgtg tctaaagagg tgttaagagg tgatgtgact 540

atgtttctta tgctcgaagg aggtggttac cacagatgcc agtttcactc cacttacaaa 600

acagagaagc cggtcgaact gcccccgaat catgtcgtag aacatcaaat tgtgaggacc 660

gaccttggcc aaagtgcaaa aggcttcacg gtcaagctgg aagcacatgc tgcggctcat 720

gttaaccctt tgaaggttca acagcaccat caccatcact aataa 765

<210> 20
<211> 765
<212> DNA
<213> Acropora aculeus

<400> 20
ttgattgatt gaaggagaaa tatcatgatc aagccatcta tcctcaacat gtctctttca 60

aagcatggca tcacacaaga aatgccgacg aaataccata tgaaaggcag tgtcaatggc 120

catgaattcg agatcgaagg tgtaggaact ggacaccctt acgaagggac acacatggcc 180

gaattagtga tcataaagcc tgcgggaaaa ccccttccat tctcctttga catactgtca 240

acagtcattc aatacggaaa cagatgcttc actaagtacc ctgcagacct gcctgactat 300

ttcaagcaag catacccagg tggaatgtca tatgaaaggt catttgtgta tcaggatgga 360

ggaattgcta cagcgagctg gaacgttagt ctcgagggaa attgcttcat ccacaaatcc 420

acctatcttg gtgtaaactt tcctgctgat ggacccgtaa tgacaaagaa gacaattggc 480

tgggataaag cctttgaaaa aatgactggg ttcaatgagg tgttaagagg tgatgtgact 540

gagtttctta tgctcgaagg aggtggttac cattcatgcc agtttcactc cacttacaaa 600

ccagagaagc cggtcgaact gcccccgaat catgtcatag aacatcacat tgtgaggacc 660

gaccttggca agactgcaaa aggcttcatg gtcaagctgg tacaacatgc tgcggctcat 720

gttaacactt tgaaggttca acatcaccat caccatcact aataa 765

<210> 21
<211> 716
<212> DNA
<213> Acropora aculeus

<400> 21
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<211> 711
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gcttgggata ttttatcacc acagtcacag tacggaagca taccattcac caaataccct 240

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cgcaaactgg atgtaaccaa tcacaacagg gattacactt ccgttgagca gcgtgaaatt 660

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<212> DNA
<213> Acropora aculeus

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caccatcact aataa 735

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<211> 711
<212> DNA
<213> Acropora hyacinthus

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cgcaaactgg atgtaaccaa tcacaacaag gattacactt ctgttgagca gcgtgaaatt 660

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<210> 27
<211> 741
<212> DNA
<213> Acropora millepora

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cctaatcatg tcgtggaaca tcacattgtg aggactgacc ttggccaaac tgcaaaaggc 660

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<213> Acropora millepora

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gagaagccgg tcacactgcc cccgaatcat gtcgtagaac atcaaattgt gaggaccgac 660

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acccctttgaa ggttcaacat caccatcacc atcactaata a 761

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<213> Acropora millepora

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<211> 744
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<211> 711
<212> DNA
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<213> Acropora nobilis

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<213> Acropora nobilis

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<210> 38
<211> 726
<212> DNA
<213> Agaricia fragilis

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ggggtgctga agggtgatgt taatatgact cttcgtgttg aaggaggtgg ccattaccga 540

gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600

atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660

gaggcagcag ttgctcgtca ttctccgctg cctaaggttg ctcatcacca tcaccatcac 720

taataa 726

<210> 39
<211> 725
<212> DNA
<213> Agaricia fragilis

<400> 39
ttgattgatt gaaggagaaa tatcatgagt gtgattgtaa aggaaatgat gactaagcta 60

cacatggaag gtactgttaa cgggcacgcc tttacaattg aaggcaaagg aaaaggcgat 120

ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc tttgccgttc 180

tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtgttcac gaagtatcca 240

gaagacatac cagacttttt caagcaggtg tttcctgaag ggtaccactg ggaaagaagt 300

attacctttg aagatcaggc cgtttgtacg gcaaccagcc acataaggct ggaccagaaa 360

gagatgtgtt ttatctatga cgtccgtttt cacggtgtga actttcccgc caatggccca 420

atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480

ggggtgctga agggtgatgt taatatgact cttcgtgttg aaggaggtgg ccattaccga 540

gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600

atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660

gaggcagcag ttgctcgtca ttctccgctg cctaaggttg ctcatcacca tcacatcact 720

aataa 725

<210> 40
<211> 726
<212> DNA
<213> Agaricia fragilis

<400> 40
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cacatggaag gtactgttaa cgggcacgcc tttacaattg aaggcaaagg aaaaggcgat 120

ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc tttgccgttc 180

tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtgttcac gaagtatcca 240

gaagacatac cagacttttt caagcaggtg tttcctgaag ggtaccactg ggaaagaagt 300

attacctttg aagatcaggc cgtttgtacg gcaaccagcc acataaggct ggaccagaaa 360

gagatgtgtt ttatctatga cgtccgtttt cacggtgtga actttcccgc caatggccca 420

atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480

ggggtgctga agggtgatgt taatatgact cttcgtgttg aaggaggtgg ccattaccga 540

gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600

atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660

ggggcagcag ttgctcgtca ttctccgctg cctaaggttt ctcatcacca tcaccatcac 720

taataa 726

<210> 41
<211> 725
<212> DNA
<213> Agaricia fragilis

<400> 41
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acatggaagg tactgttaac gggcacgcct ttacaattga aggcaaagga aaaggcgatc 120

cttacaatgg agtgcagtct atgaaccttg acgtcaaagg cggtgcgcct ttgccgttct 180

ctttcgatct cttgacgcca gcattcatgt acggcaacag agtgttcacg aagtatccag 240

aagacatacc agacttttc aagcaggtgt ttcctgaagg gtaccactgg gaaagaagta 300

ttacctttga agatcaggcc gtttgtacgg caaccagcca cataaggctg gaccagaaag 360

agatgtgttt tatctatgac gtccgttttc acggtgtgaa ctttcccgcc aatggcccaa 420

tcatgcagaa gaagatactg ggatgggagc catccactga gaaaatgtat gcacgtgatg 480

gggtgctgaa gggtgatgtt aatgtgactc ttcgtgttga aggaggtggc cattaccgag 540

ctgacttcag aactacttac aaagcaaaga agccagtcaa cctgccaggc tatcacttca 600

tagaccaccg cattgagatt accaagcaca gcaaagatta caccaatgtt gctttgtatg 660

aggcagcagt tgctcgtcat tctccgctgc ctaaggttgc tcatcaccat caccatcact 720

aataa 725

<210> 42

<211> 726
<212> DNA
<213> Agaricia fragilis

<400> 42
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cacatggaag gtactgttaa cgggcacgcc tttacaattg aaggcaaagg agagggcgat 120

ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc tttgccgttc 180

tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtgttcac gaagtatcca 240

gaagacatac cagacttttt caagcaggtg tttcctgaag ggtaccactg ggaaagaagt 300

attacctttg aagatcaggc cgtttgtacg gctaccagcc acataaggct ggaccagaaa 360

gagatgtgtt ttatctatga cgtccgtttt cacggtgtga actttcccgc caatggccca 420

atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480

ggggtgctga agggtgatgt taatatgact cttcgtgttg aaggaggtgg ccattaccga 540

gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600

atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660

ggggcagcag ttgctcgtca ttctccgctg cctaaggttg ctcatcacca tcaccatcac 720

taataa 726

<210> 43
<211> 726
<212> DNA
<213> Agaricia fragilis

<400> 43
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cacatggaag gtactgttaa cgggcacgcc tttacaattg aaggcaaagg aaaaggcgat 120

ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc tttgccgttc 180

tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtgttcac gaagtatcca 240

gaagacatac cagacttttt caagcaggtg tttcctgaag ggtaccactg ggaaagaagt 300

attacctttg aagatcaggc cgtttgtacg gcaaccagcc acataaggct ggaccagaaa 360

gagatgtgtt ttatctatga cgtccgtttt cacggtgtga actttcccgc caatggccca 420

atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480

ggggtgctga agggtgatgt taatatgact cttcgtgttg aaggaggtgg ccattaccga 540

gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600

atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660

gaggcagcag ttgctcgtca ttctccgctg cctaaggttg ctcatcacca tcaccatcac 720

taataa 726

<210> 44
<211> 726
<212> DNA
<213> Agaricia fragilis

<400> 44
ttgattgatt gaaggagaaa tatcatgagt gtgattgtaa aggaaatgat gactaagcta 60

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ccttacaatg gagtgcagtc tatgaacctt gacgtcaaag gcggtgcgcc tttgccgttc 180

tctttcgatc tcttgacgcc agcattcatg tacggcaaca gagtgttcac gaagtatcca 240

gaagacatac cagacttttt caagcaggtg tttcctgaag ggtaccactg ggaaagaagt 300

attacctttg aagatcaggc cgtttgtacg gcaaccagcc acataaggct ggaccagaaa 360

gagatgtgtt ttatctatga cgtccgtttt cacggtgtga actttcccgc caatggccca 420

atcatgcaga agaagatact gggatgggag ccatccactg agaaaatgta tgcacgtgat 480

ggggtgctga agggtgatgt taatacgact cttcgtgttg aaggaggtgg ccattaccga 540

gctgacttca gaactactta caaagcaaag aagccagtca acctgccagg ctatcacttc 600

atagaccacc gcattgagat taccaagcac agcaaagatt acaccaatgt tgctttgtat 660

gaggcagcag ttgctcgtca ttctccgctg cctaaggttg ctcatcacca tcaccatcac 720

taataa 726

<210> 45
<211> 237
<212> PRT
<213> Acropora aculeus

<400> 45

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr
1 5 10 15

Arg Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val
20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Met Ser Glu Leu Val Ile
35 40 45

Ile Lys Pro Lys Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp
65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu
85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn
100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Asn Ser Ile Phe His Gly
115 120 125

Val Asn Phe Pro Asp Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly
130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg
145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Tyr His Arg
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Glu Leu Pro
180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Ala His Ala Ala Ala His
210 215 220

Val Asn Pro Leu Lys Val Gln Gln His His His His His
225 230 235

<210> 46
<211> 237
<212> PRT
<213> Acropora aculeus

<400> 46

Met Ser Leu Ser Lys His Gly Ile Thr Gln Glu Met Pro Thr Lys Tyr
1 5 10 15

His Met Lys Gly Ser Val Asn Gly His Glu Phe Glu Ile Glu Gly Val
20 25 30

Gly Thr Gly His Pro Tyr Glu Gly Thr His Met Ala Glu Leu Val Ile
35 40 45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Thr Val Ile Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp
65 70 75 80

Leu Pro Asp Tyr Phe Lys Gln Ala Tyr Pro Gly Gly Met Ser Tyr Glu
85 90 95

Arg Ser Phe Val Tyr Gln Asp Gly Gly Ile Ala Thr Ala Ser Trp Asn
100 105 110

Val Ser Leu Glu Gly Asn Cys Phe Ile His Lys Ser Thr Tyr Leu Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Thr Lys Lys Thr Ile Gly
130 135 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Gly Phe Asn Glu Val Leu Arg
145 150 155 160

Gly Asp Val Thr Glu Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Glu Leu Pro
180 185 190

Pro Asn His Val Ile Glu His His Ile Val Arg Thr Asp Leu Gly Lys
195 200 205

Thr Ala Lys Gly Phe Met Val Lys Leu Val Gln His Ala Ala Ala His
210 215 220

Val Asn Thr Leu Lys Val Gln His His His His His His
225 230 235

<210> 47
<211> 265
<212> PRT
<213> Acropora aculeus

<400> 47

Met Thr Met Ile Thr Pro Ser Tyr Leu Gly Asp Thr Ile Glu Tyr Ser
1 5 10 15

Ser Tyr Ala Ser Asn Ala Leu Gly Ala Leu Pro Tyr Gly Arg Pro Ala
20 25 30

Gly Gly Arg Thr Ser Asp Leu Ser Tyr Ser Lys Gln Gly Ile Val Gln
35 40 45

Glu Met Lys Thr Lys Tyr Arg Met Glu Gly Ser Val Asn Gly His Glu
50 55 60

Phe Thr Ile Glu Gly Val Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln
65 70 75 80

Met Ser Glu Leu Val Ile Val Lys Pro Lys Gly Lys Pro Leu Pro Phe
85 90 95

Ser Phe Asp Ile Leu Ser Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe
100 105 110

Thr Lys Tyr Pro Ala Asp Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro
115 120 125

```
Asp Gly Met Ser Tyr Glu Arg Ser Phe Leu Phe Glu Asp Gly Ala Val
    130                 135                 140

Ala Thr Ala Ser Trp Asn Ile Arg Leu Glu Gly Asn Cys Phe Ile His
145                 150                 155                 160

Asn Ser Ile Phe His Gly Val Asn Phe Pro Ala Asp Gly Pro Val Met
                165                 170                 175

Lys Lys Lys Thr Ile Gly Trp Asp Lys Ser Phe Glu Lys Met Thr Val
            180                 185                 190

Ser Lys Glu Val Leu Arg Gly Asp Val Thr Met Phe Leu Met Leu Glu
        195                 200                 205

Gly Gly Gly Tyr His Arg Cys Gln Phe His Ser Thr Tyr Lys Thr Val
    210                 215                 220

Lys Pro Val Glu Leu Pro Pro Asn His Val Val Glu His Gln Ile Val
225                 230                 235                 240

Arg Thr Asp Leu Gly Gln Ser Ala Lys Gly Phe Thr Val Lys Leu Glu
                245                 250                 255

Ala His Ala Ala Ala His Val Thr Leu
            260                 265
```

<210> 48
<211> 237
<212> PRT
<213> Acropora aculeus

<400> 48

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Met Ser Leu Ser Lys His Gly Ile Thr Gln Glu Met Pro Thr Lys Tyr
1               5                   10                  15

His Met Lys Gly Ser Val Asn Gly His Glu Phe Glu Ile Glu Gly Val
            20                  25                  30

Gly Thr Gly His Pro Tyr Glu Gly Thr His Met Ala Glu Leu Val Ile
        35                  40                  45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
    50                  55                  60

Thr Val Ile Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp
65                  70                  75                  80

Leu Pro Asp Tyr Phe Lys Gln Ala Tyr Pro Gly Gly Met Ser Tyr Glu
                85                  90                  95

Arg Ser Phe Val Phe Gln Asp Gly Gly Ile Ala Thr Ala Ser Trp Asn
            100                 105                 110

Val Gly Leu Glu Gly Asn Cys Phe Ile His Lys Ser Thr Tyr Leu Gly
        115                 120                 125
```

```
Val Asn Phe Pro Ala Asp Gly Pro Val Met Thr Lys Lys Thr Ile Gly
    130                 135                 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Gly Phe Asn Glu Val Leu Arg
145                 150                 155                 160

Gly Asp Val Thr Glu Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser
                165                 170                 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Lys Leu Pro
            180                 185                 190

Pro Asn His Val Ile Glu His His Ile Val Arg Thr Asp Leu Gly Lys
        195                 200                 205

Thr Ala Lys Gly Phe Met Val Lys Leu Val Gln His Ala Ala Ala His
    210                 215                 220

Val Asn Pro Leu Lys Val Gln His His His His His His
225                 230                 235
```

<210> 49
<211> 237
<212> PRT
<213> Acropora aculeus

<400> 49

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Met Ser Leu Ser Lys His Gly Ile Thr Gln Glu Met Pro Thr Lys Tyr
1               5                   10                  15

His Met Lys Gly Asn Val Asn Gly His Glu Phe Glu Ile Glu Gly Val
            20                  25                  30

Gly Thr Gly His Pro Tyr Glu Gly Thr His Met Ala Glu Leu Val Ile
        35                  40                  45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
    50                  55                  60

Thr Val Ile Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp
65                  70                  75                  80

Leu Pro Asp Tyr Phe Lys Gln Ala Tyr Pro Gly Gly Met Ser Tyr Glu
                85                  90                  95

Arg Ser Phe Val Phe Gln Asp Gly Gly Ile Ala Thr Ala Ser Trp Asn
            100                 105                 110

Val Gly Leu Glu Gly Asn Cys Phe Ile His Lys Ser Thr Tyr Leu Gly
        115                 120                 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Thr Lys Lys Thr Ile Gly
    130                 135                 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Gly Phe Asn Glu Val Leu Arg
145                 150                 155                 160
```

Gly Asp Val Thr Gly Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Lys Leu Pro
180 185 190

Pro Asn His Val Ile Glu His His Ile Val Arg Thr Asp Leu Gly Lys
195 200 205

Thr Ala Lys Gly Phe Met Val Lys Leu Val Gln His Ala Ala Ala His
210 215 220

Val Asn Pro Leu Lys Val Gln His His His His His His
225 230 235

<210> 50
<211> 227
<212> PRT
<213> Acropora aculeus

<400> 50

Met Ser Val Ile Ala Lys Gln Met Thr Tyr Lys Val Tyr Met Ser Gly
1 5 10 15

Thr Val Asn Gly His Tyr Phe Glu Val Glu Gly Asp Gly Lys Gly Lys
20 25 30

Pro Tyr Glu Gly Glu Gln Thr Val Lys Leu Thr Val Thr Lys Gly Gly
35 40 45

Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Ser Gln Tyr Gly
50 55 60

Ser Ile Pro Phe Thr Lys Tyr Pro Asp Asp Ile Pro Asp Tyr Val Lys
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Thr Trp Glu Arg Ile Met Asn Phe Glu
85 90 95

Asp Gly Ala Val Cys Thr Val Ser Asn Asp Ser Ser Ile Gln Gly Asn
100 105 110

Cys Phe Ile Tyr Asn Val Lys Phe Ser Gly Leu Asn Phe Pro Pro Asn
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Gln Gly Trp Glu Pro Asn Thr Glu
130 135 140

Arg Leu Phe Ala Arg Asp Gly Met Leu Ile Gly Asn Asn Phe Met Ala
145 150 155 160

Leu Lys Leu Glu Gly Gly Gly His Tyr Leu Cys Glu Phe Lys Ser Thr
165 170 175

Tyr Lys Ala Lys Lys Pro Val Arg Met Pro Gly Tyr His Tyr Val Asp
180 185 190

Arg Lys Leu Asp Val Thr Asn His Asn Arg Asp Tyr Thr Ser Val Glu
195 200 205

Gln Arg Glu Ile Ser Ile Ala Arg Lys Pro Val Val Ala His His His
210 215 220

His His His
225

<210> 51
<211> 227
<212> PRT
<213> Acropora aculeus

<400> 51

Met Ser Val Ile Ala Lys Gln Met Thr Tyr Lys Val Tyr Met Ser Gly
1 5 10 15

Thr Val Asn Gly His Tyr Phe Glu Val Glu Gly Asp Gly Lys Gly Lys
20 25 30

Pro Tyr Glu Gly Glu Gln Thr Val Lys Leu Thr Val Thr Lys Gly Gly
35 40 45

Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Ser Gln Tyr Gly
50 55 60

Ser Ile Pro Phe Thr Lys Tyr Pro Asp Asp Ile Pro Asp Tyr Val Lys
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Thr Trp Glu Arg Ile Met Asn Phe Glu
85 90 95

Asp Gly Ala Val Cys Thr Val Ser Asn Asp Ser Ser Ile Gln Gly Asn
100 105 110

Cys Phe Ile Tyr Asn Val Lys Phe Ser Gly Leu Asn Phe Pro Pro Asn
115 120 125

Gly Pro Val Met Arg Lys Lys Thr Arg Gly Trp Glu Pro Asn Thr Glu
130 135 140

Arg Leu Phe Ala Arg Asp Gly Met Leu Ile Gly Asn Asn Phe Met Ala
145 150 155 160

Leu Lys Leu Glu Gly Gly Gly His Tyr Leu Cys Glu Phe Lys Ser Thr
165 170 175

Tyr Lys Ala Lys Lys Pro Val Arg Met Pro Gly Tyr His Tyr Val Asp
180 185 190

Arg Lys Leu Asp Val Thr Asn His Asn Arg Asp Tyr Thr Ser Val Glu
195 200 205

Gln Cys Glu Ile Ser Ile Ala Arg Lys Pro Val Val Ala His His His
210 215 220

His His His
225

<210> 52
<211> 227
<212> PRT
<213> Acropora hyacinthus

<400> 52

Met Ser Val Ile Ala Thr Gln Met Thr Tyr Lys Val Tyr Met Ser Gly
1 5 10 15

Thr Val Asn Gly His Tyr Phe Glu Val Glu Gly Asp Gly Lys Gly Lys
20 25 30

Pro Tyr Glu Gly Glu Gln Thr Val Arg Leu Thr Val Thr Lys Gly Gly
35 40 45

Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Ser Gln Tyr Gly
50 55 60

Ser Ile Pro Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Tyr Val Lys
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Thr Trp Glu Arg Ile Met Asn Phe Glu
85 90 95

Asp Gly Ala Val Cys Thr Val Ser Asn Asp Ser Ser Ile Gln Gly Asn
100 105 110

Cys Phe Ile Tyr His Val Lys Phe Ser Gly Leu Asn Phe Pro Pro Asn
115 120 125

Gly Pro Val Met Gln Lys Lys Thr Gln Gly Trp Glu Pro Asn Thr Glu
130 135 140

Arg Leu Phe Ala Arg Asp Gly Val Leu Ile Gly Asn Asn Phe Met Ala
145 150 155 160

Leu Lys Leu Glu Gly Gly Gly His Tyr Leu Cys Glu Phe Lys Ser Thr
165 170 175

Tyr Lys Ala Lys Lys Pro Val Lys Met Pro Gly Tyr His Phe Val Asp
180 185 190

Arg Lys Leu Asp Val Thr Asn His Asn Lys Asp Tyr Thr Ser Val Glu
195 200 205

Gln Arg Glu Ile Ser Ile Ala Arg Lys Pro Val Val Ala His His His
210 215 220

His His His
225

<210> 53
<211> 237

<212> PRT
<213> Acropora millepora

<400> 53

Met Ser Tyr Ser Lys Gln Gly Ile Ala Gln Val Met Lys Thr Lys Tyr
1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val
20 25 30

Gly Thr Gly Asn Pro Tyr Glu Gly Thr Gln Met Ser Glu Leu Val Ile
35 40 45

Thr Glu Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Thr Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Glu Gly
65 70 75 80

Met Thr Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Phe Glu
85 90 95

Arg Ser Phe Leu Tyr Glu Asp Gly Gly Val Ala Thr Ala Ser Trp Asn
100 105 110

Ile Arg Leu Glu Arg Asp Cys Phe Ile His Lys Ser Ile Tyr His Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly
130 135 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Val Ser Lys Asp Val Leu Arg
145 150 155 160

Gly Asp Val Thr Glu Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Thr Leu Pro
180 185 190

Pro Asn His Val Val Glu His His Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Thr Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His
210 215 220

Val Asn Pro Leu Lys Val His His His His His His His His
225 230 235

<210> 54
<211> 311
<212> PRT
<213> Acropora millepora

<400> 54

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr

1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val
20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Ile Ser Glu Leu Val Ile
35 40 45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp
65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu
85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn
100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Lys Ser Ile Phe His Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Asp
130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg
145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Ser His Arg
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Thr Leu Pro
180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Thr Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His
210 215 220

Val Ser Leu Ile Pro Arg Pro Trp Arg Pro Gly Ala Cys Asp Val Gly
225 230 235 240

Pro Asn Ser Pro Tyr Ser Glu Ser Tyr Tyr Asn Ser Leu Ala Val Val
245 250 255

Leu Gln Arg Arg Asp Trp Glu Asn Pro Gly Val Thr Gln Leu Asn Arg
260 265 270

Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala
275 280 285

Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp
290 295 300

Thr Arg Pro Val Ala Ala His
305 310

<210> 55
<211> 227
<212> PRT
<213> Acropora millepora

<400> 55

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr
1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val
20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Met Ser Glu Leu Val Ile
35 40 45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp
65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu
85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn
100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Lys Ser Ile Phe His Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Asp
130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg
145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Ser His Arg
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Thr Leu Pro
180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Thr Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His
210 215 220

Val Thr Leu
225

<210> 56
<211> 237
<212> PRT
<213> Acropora millepora

<400> 56

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr
1 5 10 15

Arg Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val
20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Met Ser Glu Leu Val Ile
35 40 45

Val Lys Pro Lys Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp
65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu
85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn
100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Asn Ser Ile Phe His Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly
130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg
145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Tyr His Arg
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Val Lys Pro Val Glu Leu Pro
180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Ala His Ala Ala Ala His
210 215 220

Val Asn Pro Leu Lys Val Gln His His His His His His
225 230 235

<210> 57
<211> 237
<212> PRT
<213> Acropora millepora

<400> 57

Met Ser His Ser Lys Gln Gly Ile Ala Gln Val Met Lys Thr Lys Tyr
1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val

20 25 30

Gly Thr Gly Asn Pro Tyr Glu Gly Ser Gln Met Ser Glu Leu Val Ile
35 40 45

Thr Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Thr Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Glu Gly
65 70 75 80

Met Thr Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu
85 90 95

Arg Ser Phe Leu Tyr Glu Asp Gly Gly Val Ala Thr Ala Ser Trp Asn
100 105 110

Ile Arg Leu Glu Arg Gly Cys Phe Ile His Lys Ser Ile Tyr His Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly
130 135 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Val Ser Lys Asp Val Leu Arg
145 150 155 160

Gly Asp Val Thr Gly Phe Leu Met Leu Glu Gly Gly Gly Tyr His Asn
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Val Thr Leu Pro
180 185 190

Pro Asn His Val Val Glu His His Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Thr Ala Lys Gly Phe Thr Ala Lys Leu Glu Glu His Ala Ala Ala His
210 215 220

Val Asn Pro Leu Lys Val Gln His His His His His His
225 230 235

<210> 58
<211> 237
<212> PRT
<213> Acropora millepora

<400> 58

Met Ser Tyr Ser Lys Gln Gly Ile Val Gln Glu Met Lys Thr Lys Tyr
1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val
20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Lys Gln Met Ser Glu Leu Val Ile
35 40 45

Ile Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser

50 55 60

Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp
65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu
85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Asn
100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Lys Ser Ile Phe His Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Asp
130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg
145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Ser His Arg
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Thr Leu Pro
180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Ala His Ala Ala Ala His
210 215 220

Val Asn Pro Leu Lys Val Lys His His His His His His
225 230 235

<210> 59
<211> 238
<212> PRT
<213> Acropora millepora

<400> 59

Met Ala Leu Ser Lys His Gly Leu Thr Lys Asp Met Thr Met Lys Tyr
1 5 10 15

His Met Glu Gly Ser Val Asp Gly His Lys Phe Val Ile Thr Gly His
20 25 30

Gly Asn Gly Asn Pro Phe Glu Gly Lys Gln Thr Met Asn Leu Cys Val
35 40 45

Val Glu Gly Gly Pro Leu Pro Phe Ser Glu Asp Ile Leu Ser Ala Thr
50 55 60

Phe Asp Tyr Gly Asn Arg Val Phe Thr Glu Tyr Pro Gln Gly Met Val
65 70 75 80

Asp Phe Phe Lys Asn Ser Cys Pro Ala Gly Tyr Thr Trp His Arg Ser

85 90 95

Leu Leu Phe Glu Asp Gly Ala Val Cys Thr Thr Ser Ala Asp Ile Thr
100 105 110

Val Ser Val Glu Glu Asn Cys Phe Tyr His Asn Ser Lys Phe His Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Met Thr Thr Asn
130 135 140

Trp Glu Pro Ser Cys Glu Lys Ile Ile Pro Val Pro Arg Gln Gly Ile
145 150 155 160

Leu Lys Gly Asp Ile Ala Met Tyr Leu Leu Leu Lys Asp Gly Gly Arg
165 170 175

Tyr Arg Cys Gln Phe Asp Thr Ile Tyr Lys Ala Lys Ser Asp Pro Lys
180 185 190

Glu Met Pro Glu Trp His Phe Ile Gln His Lys Leu Thr Arg Glu Asp
195 200 205

Arg Ser Asp Ala Lys Asn Gln Lys Trp Gln Leu Val Glu His Ala Val
210 215 220

Ala Ser Arg Ser Ala Leu Pro Gly His His His His His His
225 230 235

<210> 60
<211> 227
<212> PRT
<213> Acropora millepora

<400> 60

Met Ser Val Ile Ala Lys Gln Met Thr Tyr Lys Val Tyr Met Ser Gly
1 5 10 15

Thr Val Asn Gly His Tyr Phe Glu Val Glu Gly Asp Gly Lys Gly Lys
20 25 30

Pro Tyr Glu Gly Glu Gln Thr Val Lys Leu Thr Val Thr Lys Gly Gly
35 40 45

Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Cys Gln Tyr Gly
50 55 60

Ser Ile Pro Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Tyr Val Lys
65 70 75 80

Gln Ser Phe Pro Glu Gly Tyr Thr Trp Glu Arg Ile Met Asn Phe Glu
85 90 95

Asp Gly Ala Val Cys Thr Val Ser Asn Asp Ser Ser Ile Gln Gly Asn
100 105 110

Cys Phe Ile Tyr His Val Lys Phe Ser Gly Leu Asn Phe Pro Pro Asn

115 120 125

Gly Pro Val Met Gln Lys Lys Thr Gln Gly Trp Glu Pro Asn Thr Glu
130 135 140

Arg Leu Phe Ala Arg Asp Gly Met Leu Leu Gly Asn Asn Phe Met Ala
145 150 155 160

Leu Lys Leu Glu Gly Gly Gly His Tyr Leu Cys Glu Phe Lys Thr Thr
165 170 175

Tyr Lys Ala Lys Lys Pro Val Lys Met Pro Gly Tyr His Tyr Val Asp
180 185 190

Arg Lys Leu Asp Val Thr Asn His Asn Lys Asp Tyr Thr Ser Val Glu
195 200 205

Gln Cys Glu Ile Ser Ile Ala Arg Lys Pro Val Val Ala His His His
210 215 220

His His His
225

<210> 61
<211> 237
<212> PRT
<213> Acropora nobilis

<400> 61

Met Ser Tyr Ser Lys Gln Gly Ile Ala Gln Val Met Lys Thr Lys Tyr
1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val
20 25 30

Gly Thr Gly Asn Pro Tyr Glu Gly Thr Gln Met Ser Glu Leu Val Ile
35 40 45

Thr Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Thr Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Glu Gly
65 70 75 80

Met Thr Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Cys Glu
85 90 95

Arg Ser Phe Leu Tyr Glu Asp Gly Gly Val Ala Thr Ala Ser Trp Asn
100 105 110

Ile Arg Leu Glu Arg Asp Cys Phe Ile His Lys Ser Ile Tyr His Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly
130 135 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Val Ser Lys Asp Val Leu Arg

145 150 155 160

Gly Asp Val Thr Glu Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Ala Ala Leu Pro
180 185 190

Pro Asn His Val Val Glu His His Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His
210 215 220

Val Asn Pro Leu Lys Val Gln His His His His His His
225 230 235

<210> 62
<211> 237
<212> PRT
<213> Acropora nobilis

<400> 62

Met Ser Tyr Ser Lys Gln Gly Ile Ala Gln Val Met Lys Thr Lys Tyr
1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Ile Glu Gly Val
20 25 30

Gly Thr Gly Asn Pro Tyr Glu Gly Thr Gln Met Ser Glu Leu Val Ile
35 40 45

Thr Lys Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Thr Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Glu Gly
65 70 75 80

Met Thr Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu
85 90 95

Arg Ser Phe Leu Tyr Glu Asp Gly Gly Val Ala Thr Ala Gly Trp Asn
100 105 110

Ile Arg Leu Glu Arg Asp Cys Phe Ile His Lys Ser Ile Tyr His Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Lys Lys Lys Thr Ile Gly
130 135 140

Trp Asp Lys Ala Phe Glu Lys Met Thr Val Ser Lys Asp Val Leu Arg
145 150 155 160

Gly Asp Val Thr Gly Phe Leu Met Leu Glu Gly Gly Gly Tyr His Ser
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Pro Glu Lys Pro Ala Ala Leu Pro

180 185 190

Pro Asn His Val Val Glu His His Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Glu His Ala Ala Ala His
210 215 220

Val Asn Pro Leu Lys Val Gln His His His His His His
225 230 235

<210> 63
<211> 237
<212> PRT
<213> Acropora nobilis

<400> 63

Met Ser Tyr Ser Lys Gln Gly Ile Ala Gln Glu Met Lys Thr Lys Tyr
1 5 10 15

His Met Glu Gly Ser Val Asn Gly His Glu Phe Thr Val Glu Gly Val
20 25 30

Gly Thr Gly Tyr Pro Tyr Glu Gly Glu Gln Met Ser Glu Leu Val Ile
35 40 45

Ile Glu Pro Ala Gly Lys Pro Leu Pro Phe Ser Phe Asp Ile Leu Ser
50 55 60

Ser Val Phe Gln Tyr Gly Asn Arg Cys Phe Thr Lys Tyr Pro Ala Asp
65 70 75 80

Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro Asp Gly Met Ser Tyr Glu
85 90 95

Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Ala Thr Ala Ser Trp Lys
100 105 110

Ile Arg Leu Glu Gly Asn Cys Phe Ile His Asn Ser Ile Phe Asn Gly
115 120 125

Val Asn Phe Pro Ala Asp Gly Pro Val Met Glu Lys Lys Thr Ile Gly
130 135 140

Trp Asp Lys Ser Phe Glu Lys Met Thr Val Ser Lys Glu Val Leu Arg
145 150 155 160

Gly Asp Val Thr Met Phe Leu Met Leu Glu Gly Gly Gly Ser His Arg
165 170 175

Cys Gln Phe His Ser Thr Tyr Lys Thr Glu Lys Pro Val Thr Leu Pro
180 185 190

Pro Asn His Val Val Glu His Gln Ile Val Arg Thr Asp Leu Gly Gln
195 200 205

Ser Ala Lys Gly Phe Thr Val Lys Leu Glu Ala His Ala Ala Ala His

210 215 220

Val Asn Pro Leu Lys Val Lys His His His His His His
225 230 235

<210> 64
<211> 232
<212> PRT
<213> Agaricia fragilis

<400> 64

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly
1 5 10 15

Thr Val Asn Gly His Ala Leu Thr Ile Glu Gly Lys Gly Lys Gly Asp
20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala
35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly
50 55 60

Asn Arg Val Phe Ala Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys
65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu
85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys
100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro
115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser
130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn
145 150 155 160

Met Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg
165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe
180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn
195 200 205

Val Ala Leu Tyr Glu Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys
210 215 220

Val Ala His His His His His His
225 230

<210> 65
<211> 306
<212> PRT

<213> Agaricia fragilis

<400> 65

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly
1 5 10 15

Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Lys Gly Asp
20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala
35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys
65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu
85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys
100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro
115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser
130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn
145 150 155 160

Met Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg
165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe
180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn
195 200 205

Val Ala Leu Tyr Glu Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys
210 215 220

Val Ala His His His His Ile Thr Asn Lys Ser Arg Gly His Gly Gly
225 230 235 240

Arg Glu His Ala Thr Ser Gly Pro Ile Arg Pro Ile Val Ser Arg Ile
245 250 255

Thr Ile His Trp Pro Ser Phe Tyr Asn Val Val Thr Gly Lys Thr Leu
260 265 270

Ala Leu Pro Asn Leu Ile Ala Leu Gln His Ile Pro Leu Ser Pro Ala
275 280 285

Gly Val Ile Ala Lys Arg Pro Ala Pro Ile Ala Leu Pro Asn Ser Cys
290 295 300

Ala Ala
305

<210> 66
<211> 232
<212> PRT
<213> Agaricia fragilis

<400> 66

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly
1 5 10 15

Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Lys Gly Asp
20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala
35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys
65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu
85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys
100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro
115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser
130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn
145 150 155 160

Met Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg
165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe
180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn
195 200 205

Val Ala Leu Tyr Gly Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys
210 215 220

Val Ser His His His His His His
225 230

<210> 67
<211> 232
<212> PRT
<213> Agaricia fragilis

<400> 67

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly
1 5 10 15

Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Lys Gly Asp
20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala
35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys
65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu
85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys
100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro
115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser
130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn
145 150 155 160

Val Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg
165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe
180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn
195 200 205

Val Ala Leu Tyr Glu Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys
210 215 220

Val Ala His His His His His His
225 230

<210> 68
<211> 232

<212> PRT
<213> Agaricia fragilis

<400> 68

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly
1 5 10 15

Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Glu Gly Asp
20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala
35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys
65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu
85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys
100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro
115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser
130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn
145 150 155 160

Met Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg
165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe
180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn
195 200 205

Val Ala Leu Tyr Gly Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys
210 215 220

Val Ala His His His His His His
225 230

<210> 69
<211> 232
<212> PRT
<213> Agaricia fragilis

<400> 69

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly

1 5 10 15

Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Lys Gly Asp
20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala
35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys
65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu
85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys
100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro
115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser
130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn
145 150 155 160

Met Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg
165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe
180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn
195 200 205

Val Ala Leu Tyr Glu Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys
210 215 220

Val Ala His His His His His His
225 230

<210> 70
<211> 232
<212> PRT
<213> Agaricia fragilis

<400> 70

Met Ser Val Ile Val Lys Glu Met Met Thr Lys Leu His Met Glu Gly
1 5 10 15

Thr Val Asn Gly His Ala Phe Thr Ile Glu Gly Lys Gly Lys Gly Asp
20 25 30

Pro Tyr Asn Gly Val Gln Ser Met Asn Leu Asp Val Lys Gly Gly Ala

35 40 45

Pro Leu Pro Phe Ser Phe Asp Leu Leu Thr Pro Ala Phe Met Tyr Gly
50 55 60

Asn Arg Val Phe Thr Lys Tyr Pro Glu Asp Ile Pro Asp Phe Phe Lys
65 70 75 80

Gln Val Phe Pro Glu Gly Tyr His Trp Glu Arg Ser Ile Thr Phe Glu
85 90 95

Asp Gln Ala Val Cys Thr Ala Thr Ser His Ile Arg Leu Asp Gln Lys
100 105 110

Glu Met Cys Phe Ile Tyr Asp Val Arg Phe His Gly Val Asn Phe Pro
115 120 125

Ala Asn Gly Pro Ile Met Gln Lys Lys Ile Leu Gly Trp Glu Pro Ser
130 135 140

Thr Glu Lys Met Tyr Ala Arg Asp Gly Val Leu Lys Gly Asp Val Asn
145 150 155 160

Thr Thr Leu Arg Val Glu Gly Gly Gly His Tyr Arg Ala Asp Phe Arg
165 170 175

Thr Thr Tyr Lys Ala Lys Lys Pro Val Asn Leu Pro Gly Tyr His Phe
180 185 190

Ile Asp His Arg Ile Glu Ile Thr Lys His Ser Lys Asp Tyr Thr Asn
195 200 205

Val Ala Leu Tyr Glu Ala Ala Val Ala Arg His Ser Pro Leu Pro Lys
210 215 220

Val Ala His His His His His His
225 230

<210> 71
<211> 717
<212> DNA
<213> Acropora aculeus

<400> 71
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agtgtcaatg gccatgaatt cacgatcgaa ggtgtaggaa ctgggtaccc ttacgaaggg 120

aaacagatgt ccgaattagt gatcatcaag cctaagggaa agccccttcc attctccttt 180

gacatactgt catcagtctt tcaatatgga aacaggtgct tcacaaagta ccctgcagac 240

atgcctgact atttcaagca agcattccca gatggaatgt catatgaaag gtcatttcta 300

tttgaggatg gagcagttgc tacagccagc tggaacattc gtctcgaagg aaattgcttc 360

atccacaatt ccatctttca tggcgtaaac tttcccgatg atggacccgt aatgaaaaag 420

aagacaattg gctgggataa gtccttcgaa aaaatgactg tgtctaaaga ggtgttaaga 480

ggtgatgtga ctatgtttct tatgctcgaa ggaggtggtt accacagatg ccagtttcac 540

tccacttaca aaacagagaa gccggtcgaa ctgcccccga atcatgtcgt agaacatcaa 600

attgtgagga ccgaccttgg ccaaagtgca aaaggcttca cggtcaagct ggaagcacat 660

gctgcggctc atgttaaccc tttgaaggtt caacagcacc atcaccatca ctaataa 717

<210> 72
<211> 717
<212> DNA
<213> Acropora aculeus

<400> 72
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acacacatgg ccgaattagt gatcataaag cctgcgggaa aacccccttcc attctccttt 180

gacatactgt caacagtcat tcaatacgga aacagatgct tcactaagta ccctgcagac 240

ctgcctgact atttcaagca agcataccca ggtggaatgt catatgaaag gtcatttgtg 300

tatcaggatg gaggaattgc tacagcgagc tggaacgtta gtctcgaggg aaattgcttc 360

atccacaaat ccacctatct tggtgtaaac tttcctgctg atggacccgt aatgacaaag 420

aagacaattg gctgggataa agcctttgaa aaaatgactg ggttcaatga ggtgttaaga 480

ggtgatgtga ctgagtttct tatgctcgaa ggaggtggtt accattcatg ccagtttcac 540

tccacttaca aaccagagaa gccggtcgaa ctgcccccga atcatgtcat agaacatcac 600

attgtgagga ccgaccttgg caagactgca aaaggcttca tggtcaagct ggtacaacat 660

gctgcggctc atgttaacac tttgaaggtt caacatcacc atcaccatca ctaataa 717

<210> 73
<211> 665
<212> DNA
<213> Acropora aculeus

<400> 73
atggaaggca gtgtcaatgg ccatgaattc acgatcgaag gtgtaggaac tgggtaccct 60

tacgaaggga agcagatgtc cgaattagtg atcgtcaagc ctaagggaaa gccccttcca 120

ttctcctttg acatactgtc atcagtcttt caatatggaa acaggtgctt cacaaagtac 180

cctgcagaca tgcctgacta tttcaagcaa gcattcccag atggaatgtc atatgaaagg 240

tcatttctat ttgaggatgg agcagttgct acagccagct ggaacattcg tctcgaagga 300

aattgcttca tccacaattc catctttcat ggcgtaaact ttcccgctga tggacccgta 360

atgaaaaaga agacaattgg ctgggataag tccttcgaaa aaatgactgt gtctaaagag 420

gtgttaagag gtgatgtgac tatgtttctt atgctcgaag gaggtggtta ccacagatgc 480

cagtttcact ccacttacaa aacagtgaag ccggtcgaac tgcccccgaa tcatgtcgta 540

gaacatcaaa ttgtgaggac cgaccttggc caaagtgcaa aaggcttcac agtcaagctg 600

gaagcacatg ctgcggctca tgtaaccctt tgaaggttca acatcaccat caccatcact 660

aataa 665

<210> 74
<211> 717
<212> DNA
<213> Acropora aculeus

<400> 74
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agtgtcaatg gccatgaatt cgagatcgaa ggtgtaggaa ctggacaccc ttacgaaggg 120

acacacatgg ccgaattagt gatcataaag cctgcgggaa aacccccttcc attctccttt 180

gacatactgt caacagtcat tcaatacgga aacagatgct tcactaagta ccctgcagac 240

ctgcctgact atttcaagca agcataccca ggtggaatgt catatgaaag gtcatttgta 300

tttcaggatg gaggaattgc tacagcgagc tggaacgtcg gtctcgaggg aaattgcttc 360

atccacaaat ccacctatct tggtgtaaac tttcctgctg atggacccgt aatgacaaag 420

aagacaattg gctgggataa agcctttgaa aaaatgactg ggttcaatga ggtgttaaga 480

ggtgatgtga ctgagtttct tatgctcgaa ggaggtggtt accattcatg ccagtttcac 540

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attgtgagga ccgaccttgg caagactgca aaaggcttca tggtcaagct ggtacaacat 660

gctgcggctc atgttaaccc tttgaaggtt caacatcacc atcaccatca ctaataa 717

<210> 75
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<212> DNA
<213> Acropora aculeus

<400> 75
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gacatactgt caacagtcat tcaatacgga aacagatgct tcactaagta ccctgcagac 240

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tttcaggatg gaggaattgc tacagcgagc tggaacgttg gtctcgaggg aaattgcttc 360

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aagacaattg gctgggataa agcctttgaa aaaatgactg ggttcaatga ggtgttaaga 480

ggcgatgtga ctgggtttct tatgctcgaa ggaggtggtt accattcatg ccagtttcac 540

tccacttaca aaccagagaa gccggtcaaa ctgcccccga atcatgtcat agaacatcac 600

attgtgagga ccgaccttgg caagactgca aaaggcttca tggtcaagct ggtacaacat 660

gctgcggctc atgtgaaccc tttgaaggtt caacatcacc atcaccatca ctaataa 717

<210> 76
<211> 666
<212> DNA
<213> Acropora aculeus

<400> 76
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gcacgagatg gaatgctgat aggaaacaac tttatggctc tgaagttaga aggaggtggt 480

cactatttgt gtgaattcaa atctacttac aaggcaaaga agcctgtgag gatgccaggg 540

tatcactatg ttgaccgcaa actggatgta accaatcaca acagggatta cacttccgtt 600

gagcagcgtg aaatttccat tgcacgcaaa cctgtggtcg cccatcacca tcaccatcac 660

taataa 666

<210> 77
<211> 687
<212> DNA
<213> Acropora aculeus

<400> 77
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aacagggatt acacttccgt tgagcagtgt gaaatttcca ttgcacgcaa acctgtggtc 660

gcccatcacc atcaccatca ctaataa 687

<210> 78
<211> 666
<212> DNA
<213> Acropora hyacinthus

<400> 78
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gagcagcgtg aaatttccat tgcacgcaaa cctgtggtcg cccaccacca tcaccatcac 660

taataa 666

<210> 79
<211> 684
<212> DNA
<213> Acropora millepora

<400> 79
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ggcttcacag tcaagctgga agaacatgct gcggctcatg ttaacccttt gaaggttcac 660

catcaccatc accatcacta ataa 684

<210> 80
<211> 681
<212> DNA
<213> Acropora millepora

<400> 80
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aaacagatat ccgaattagt gatcatcaag cctgcgggaa aacccccttcc attctccttt 180

gacatactgt catcagtctt tcaatatgga aacaggtgct tcacaaagta ccctgcagac 240

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tccacttaca aaacagagaa gccggtcaca ctgcccccga atcatgtcgt agaacatcaa 600

attgtgagga ccgaccttgg ccaaactgca aaaggcttca cagtcaagct ggaagaacat 660

gctgcggctc atgttagcct a 681

<210> 81
<211> 684
<212> DNA

<213> Acropora millepora

<400> 81
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aaacagatgt ccgaattagt gatcatcaag cctgcgggaa aacccccttcc attctccttt 180

gacatactgt catcagtctt tcaatatgga aacaggtgct tcacaaagta ccctgcagac 240

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tccacttaca aaacagagaa gccggtcaca ctgcccccga atcatgtcgt agaacatcaa 600

attgtgagga ccgaccttgg ccaaactgca aaaggcttca cagtcaagct ggaagaacat 660

gctgcggctc atgtaaccct ttga 684

<210> 82
<211> 717
<212> DNA
<213> Acropora millepora

<400> 82
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agtgtcaatg gccatgaatt cacgatcgaa ggtgtaggaa ctgggtaccc ttacgaaggg 120

aagcagatgt ccgaattagt gatcgtcaag cctaagggaa agccccttcc attctccttt 180

gacatactgt catcagtctt tcaatatgga aacaggtgct tcacaaagta ccctgcagac 240

atgcctgact atttcaagca agcattccca gatggaatgt catatgaaag gtcatttcta 300

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ggtgatgtga ctatgtttct tatgctcgaa ggaggtggtt accacagatg ccagtttcac 540

tccacttaca aaacagtgaa gccggtcgaa ctgcccccga atcatgtcgt agaacatcaa 600

attgtgagga ccgaccttgg ccaaagtgca aaaggcttca cagtcaagct ggaagcacat 660

gctgcggctc atgttaaccc tttgaaggtt caacatcacc atcaccatca ctaataa 717

<210> 83
<211> 717
<212> DNA
<213> Acropora millepora

<400> 83
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agtgtcaatg gccatgaatt cacgatcgaa ggtgtaggaa ctggaaaccc ttacgaaggc 120

tcacagatgt ccgagttagt gatcaccaag cctgcaggaa aacccttcc attctccttt 180

gacattctct caacagtctt tcaatatgga aacaggtgct tcacaaagta ccctgaagga 240

atgactgact atttcaagca agcattccca gatggaatgt catatgaaag gtcatttcta 300

tatgaggatg gaggagttgc tacagccagc tggaacattc gtcttgagag aggttgcttc 360

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attgtgagga ctgaccttgg ccaaactgca aaaggcttca cagccaagct ggaagaacat 660

gctgcggctc atgtaaaccc tttgaaggtt caacatcacc atcaccatca ctaataa 717

<210> 84
<211> 684
<212> DNA
<213> Acropora millepora

<400> 84
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gtaggaactg ggtaccctta cgaagggaaa cagatgtccg aattagtgat catcaagcct 120

gcgggaaaac cccttccatt ctcctttgac atactgtcat cagtctttca atatggaaac 180

aggtgcttca caaagtaccc tgcagacatg cctgactatt tcaagcaagc attcccagat 240

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cccccgaatc atgtcgtaga acatcaaatt gtgaggaccg accttggcca aagtgcaaaa 600

ggctttacag tcaagctgga agcacatgct gcggctcatg ttaacccttt gaaggttaaa 660

catcaccatc accatcacta ataa 684

<210> 85
<211> 687
<212> DNA
<213> Acropora millepora

<400> 85
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cacggcaatg gaaatccttt cgaagggaaa cagactatga atctgtgtgt ggttgaaggg 120

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gctaagaacc agaaatggca actggtagaa catgctgttg cttcccgatc cgcattgccc 660

ggacatcacc atcaccatca ctaataa 687

<210> 86
<211> 666
<212> DNA
<213> Acropora millepora

<400> 86
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tatcactatg ttgaccgcaa actggatgta accaatcaca acaaggatta cacttcggtt 600

gagcagtgtg aaatttccat tgcacgcaaa cctgtggtcg cccatcacca tcaccatcac 660

taataa 666

<210> 87
<211> 717
<212> DNA
<213> Acropora nobilis

<400> 87
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acacagatgt ccgaattagt gatcaccaag cctgcaggaa aacccccttcc attctccttt 180

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tatgaggatg gaggagttgc tacagccagc tggaacattc gtcttgagag agattgcttc 360

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attgtgagga ctgaccttgg ccaaagtgca aaaggcttca cagtcaagct ggaagaacat 660

gctgcggctc atgttaaccc tttgaaggtt caacatcacc atcaccatca ctaataa 717

<210> 88
<211> 684
<212> DNA
<213> Acropora nobilis

<400> 88
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ggtggttacc acagctgcca gtttcactcc acttacaaac cagaaaagcc ggctgcactg 540

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catcaccatc accatcacta ataa 684

<210> 89
<211> 684
<212> DNA
<213> Acropora nobilis

<400> 89
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ggctttacag tcaagctgga agcacatgct gcggctcatg ttaacccttt gaaggttaaa 660

catcaccatc accatcacta ataa 684

<210> 90
<211> 681
<212> DNA
<213> Agaricia fragilis

<400> 90
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ccaggctatc acttcataga ccaccgcatt gagattacca agcacagcaa agattacacc 600

aatgttgctt tgtatgaggc agcagttgct cgtcattctc cgctgcctaa ggttgctcat 660

caccatcacc atcactaata a 681

<210> 91
<211> 680
<212> DNA
<213> Agaricia fragilis

<400> 91
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<210> 92
<211> 681
<212> DNA
<213> Agaricia fragilis

<400> 92
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gcgcctttgc cgttctcttt cgatctcttg acgccagcat tcatgtacgg caacagagtg 180

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aatgttgctt tgtatggggc agcagttgct cgtcattctc cgctgcctaa ggtttctcat 660

caccatcacc atcactaata a 681

<210> 93
<211> 678
<212> DNA
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

REVISED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 March 2005 (03.03.2005)

PCT

(10) International Publication Number
WO 2005/019252 A2

(51) International Patent Classification[7]: C07K 14/37, C12N 15/62, A61K 49/00, C12N 15/12

(21) International Application Number: PCT/US2004/016252

(22) International Filing Date: 20 May 2004 (20.05.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/472,196 22 May 2003 (22.05.2003) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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Published:
— *with declaration under Article 17(2)(a); without abstract; title not checked by the International Searching Authority*

(48) Date of publication of this revised version: 28 July 2005

(15) Information about Correction:
see PCT Gazette No. 30/2005 of 28 July 2005, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL FLUORESCENT AND COLORED PROTEINS, AND POLYNUCLEOTIDES THAT ENCODE THESE PROTEINS

(57) Abstract:

WO 2005/019252 A2

Applicant's or agent's file reference	IMPORTANT DECLARATION	Date of mailing *(day/month/year)*
UF-364XC1 PCT		14/03/2005
International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/US2004/016252	20/05/2004	22/05/2003
International Patent Classification (IPC) or both national classification and IPC		
Applicant		
UNIVERSITY OF FLORIDA RESEARCH FOUNDATION, INC.		

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below

1. ☐ The subject matter of the international application relates to:
   - a. ☐ scientific theories.
   - b. ☐ mathematical theories
   - c. ☐ plant varieties.
   - d. ☐ animal varieties.
   - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
   - f. ☐ schemes, rules or methods of doing business.
   - g. ☐ schemes, rules or methods of performing purely mental acts.
   - h. ☐ schemes, rules or methods of playing games.
   - i. ☐ methods for treatment of the human body by surgery or therapy.
   - j. ☐ methods for treatment of the animal body by surgery or therapy.
   - k. ☐ diagnostic methods practised on the human or animal body.
   - l. ☐ mere presentations of information.
   - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.
2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:
   ☐ the description ☐ the claims ☐ the drawings
3. ☒ The failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions prevents a meaningful search from being carried out:
   ☐ the written form has not been furnished or does not comply with the standard.
   ☒ the computer readable form has not been furnished or does not comply with the standard.
4. ☐ The failure of the tables related to the nucleotide and/or amino acid sequence listing to comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions prevents a meaningful search from being carried out:
   ☐ the written form has not been furnished.
   ☐ the computer readable form has not been furnished or does not comply with the technical requirements.
5. Further comments: The requested Sequence Listing on floppy was not furnished

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Henriëtte Huysing-Solles

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The claims of the underlying application have, according to PCT Rule 13ter.1.c, not been searched since the Sequence Listing has not been furnished in machine readable form as prescribed in the administrative instructions under Rule 5:2. The applicant has not remedied the disclosed deficiencies within the time limit fixed in the invitation pursuant to PCT Rule 13ter.1.a.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

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